EVALUATION OF RBC ACETYLCHOLINESTERASE POINT OF CARE TESTING USING ACHE RAPID CHECK MOBILE IN PATIENTS WITH ORGANOPHOSPHATE POISONING AND ITS CORRELATION WITH CLINICAL PROFILE

.

A dissertation submitted in partial fulfilment of the rules and regulations for MD General Medicine examination of the Tamil Nadu Dr.M.G.R Medical University, Chennai, to be held in April 2017

DECLARATION

This is to declare that this dissertation titled — "Evaluation of RBC acetylcholinesterase point of care testing using ACHE rapid check mobile in patients with organophosphate poisoning and its correlation with clinical profile" is my original work done in partial fulfilment of rules and regulations for MD General Medicine examination of the Tamil Nadu Dr.M.G.R Medical University, Chennai to be held in April 2017.

CANDIDATE

Aneez Joseph

Post graduate Registrar

General Medicine

Christian Medical College

Vellore

CERTIFICATE

This is to certify that the dissertation entitled,"Evaluation of RBC acetylcholinesterase point of care testing using ACHE rapid check mobile in patients with organophosphate poisoning and its correlation with clinical profile" a bonafide work done by Dr. Aneez Joseph towards the partial fulfilment of rules and regulations for MD General Medicine degree examination of the Tamil Nadu Dr.M.G.R Medical University, to be conducted in April 2017.

GUIDE

Dr.Anand Zachariah

Professor and Head of the Department

Department of Medicine

Christian Medical College

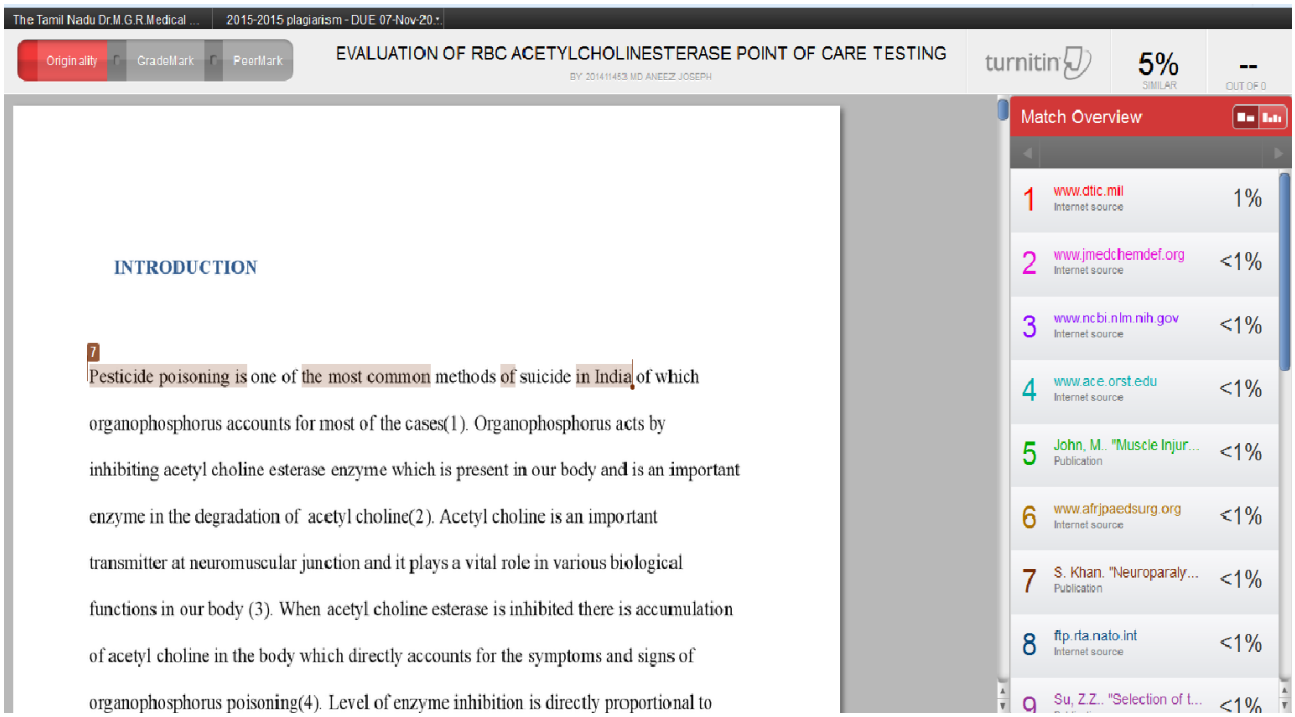
Vellore

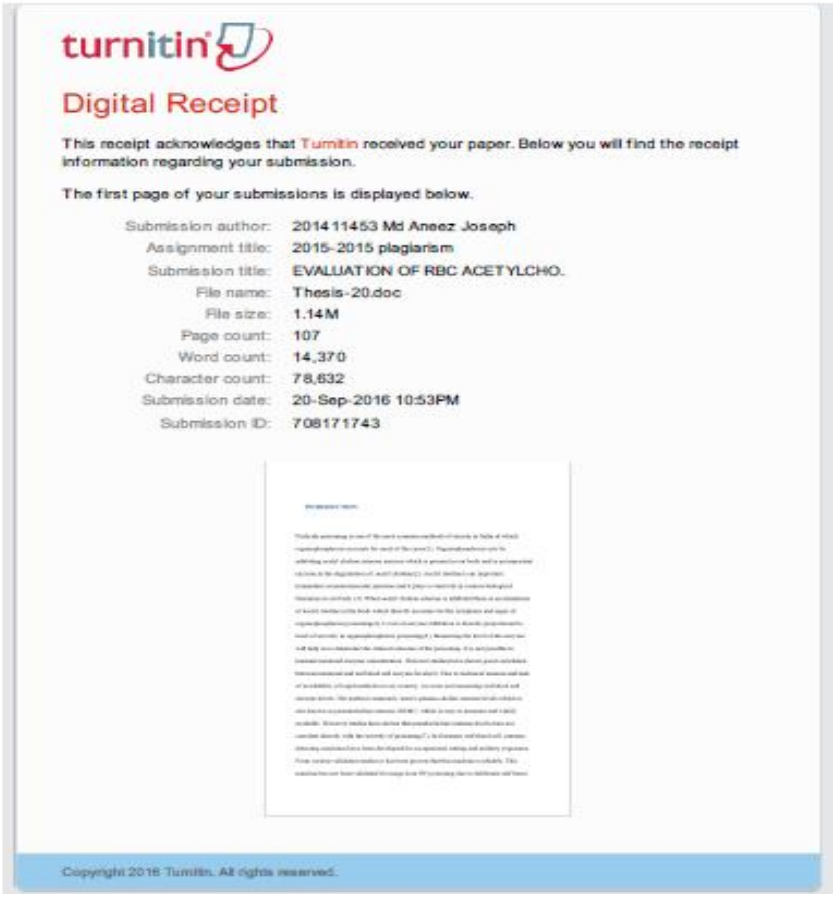
CERTIFICATE

This is to certify that the dissertation entitled,"Evaluation of RBC acetylcholinesterase point of care testing using ACHE rapid check mobile in patients with organophosphate poisoning and its correlation with clinical profile" a bonafide work done by Dr. Aneez Joseph towards the partial fulfilment of rules and regulations for MD General Medicine degree examination of the Tamil Nadu Dr.M.G.R Medical University, to be conducted in April 2017.

PRINCIPAL	HEAD OF THE DEPARTMENT
Dr.Anna B Pulimood	Dr.Anand Zachariah
Professor	Professor and Head
Department of Pathology	Department of Medicine
Christian Medical College	Christian Medical College
Vellore	Vellore

PLAGIARISM CERTIFICATE





ACKNOWLEDGEMENTS

I would like to gratefully acknowledge various people who have been with me for the last 3 years to complete this thesis dissertation .

First of all I would like to thank *God almighty* for this opportunity and by whose grace this was possible.

Secondly, I would like to thank my supervisor and guide Dr.Anand Zachariah, Professor and Head of the department of Medicine, who has encouraged and challenged me with novel ideas and who has been a great mentor throughout my journey.

I owe an enormous debt of gratitude to Dr.*Joe Fleming*, Professor of Clinical Biochemistry and Mr. *Arun Jose*, Senior Lecturer, Clinical Biochemistry for conducting the cholinesterase studies.I would like to express my sincere gratitude to Mr.*Arthur* and his team of respiratory technicians for assisting with sample collection.

I thank Dr.*Horst Thiermann* and Dr.*Franz Worek*, Institut für Pharmakologie und Toxikologie der Bundeswehr, Germany for the for providing the standard operating protocol for the tests. I thank Miss.*Thenmozhi* and Dr. Jeyaseelan, department of Biostatistics, for his expertise in statistical analysis.

Special thanks to my friends and colleagues *Dr. Asisha Janeela* and *Dr. Sandhya Suresh* who helped with patient recruitment.

Lastly I would like to express my profound gratitude to my husband Jithin Mathew and my family for providing me unconditional support and continuous encouragement throughout the process of the thesis.

CONTENTS

TABLE OF TABLES

TABLE OF FIGURES

INTRODUCTION

Pesticide poisoning is one of the most common methods of suicide in India of which organophosphorus accounts for most of the cases(1). Organophosphorus acts by inhibiting acetyl choline esterase enzyme which is present in our body and is an important enzyme in the degradation of acetyl choline(2). Acetyl choline is an important transmitter at neuromuscular junction and it plays a vital role in various biological functions in our body (3). When acetyl choline esterase is inhibited there is accumulation of acetyl choline in the body which directly accounts for the symptoms and signs of organophosphorus poisoning(4). Level of enzyme inhibition is directly proportional to level of severity in organophosphorus poisoning(5). Measuring the level of the enzyme will help us to determine the clinical outcome of the poisoning. It is not possible to measure neuronal enzyme concentration. However studies have shown good correlation between neuronal and red blood cell enzyme levels(6). Due to technical reasons and lack of availability of rapid methods in our country, we were not measuring red blood cell enzyme levels. The method commonly used is plasma choline esterase levels which is also known as pseudocholine esterase (BChE) which is easy to measure and widely available. However studies have shown that pseudocholine esterase levels does not correlate directly with the severity of poisoning(7). In Germany red blood cell esterase detecting machines have been developed for occupational setting and military exposures. From various validation studies it has been proven that this machine is reliable. This machine has not been validated for mega dose OP poisoning due to deliberate self-harm. Given the scale of pesticide poisoning in India and the lack of BChE measurement in most hospitals, this

machine has potential for wide use in diagnosing OP poisoning, assessing severity of poisoning and predicting the clinical outcome(8). Thus this study has been planned to measure red blood cell esterase level in patients with organophosphorus poisoning with rapid check mobile machine along with simultaneous lab measurements of the blood level of the enzyme. Serial measurement of the enzyme will be correlated to the temporal clinical profile of the poisoning.

The proposed research study assesses the utility of RBC rapid check mobile in detecting the acetyl choline esterase concentration in the blood of patients as a diagnostic test for acute Organophosphorus poisoning.

AIMS

To validate RBC AChE Rapid Check mobile in detection of RBC AChE in patients with acute OP poisoning and determine its role in predicting clinical outcomes.

OBJECTIVES

1. To validate RBC AChE check mobile for rapid detection of RBC AChE in patients of acute OP poisoning.

2. To determine admission levels of RBC AChE and blood BChE and their correlation to severity of OP poisoning, cholinergic crises, need for mechanical ventilation and development of intermediate syndrome in patients of acute OP poisoning.

3. To determine the temporal profile of RBC AChE and blood BChE and their correlation to duration of mechanical ventilation, duration of intermediate syndrome and of hospitalization in patients of acute OP poisoning.

LITERATURE REVIEW

Deliberate self harm (DSH) is one of the common causes of death worldwide. The WHO estimated in 2012 that it is the 15 th leading cause of death. Eight lakh people die due to suicide every year worldwide that is equivalent to 1 person every 40 seconds. This accounts for 1.4% of world population(9). DSH can occur at any age, but is more common in 15-29 years of age and it is the second most common cause of death in this age group. Even though suicide occurs worldwide it is more common in low and middle income countries which accounts for 75% of total cases. (10)

SUICIDE IN INDIA

Age standardized suicide rate per 100000 population is greater than 15 in India which makes the country fall into the category of countries with high suicide rate and mortality(10).

According to WHO 2012 estimates DSH is one of the top 10 causes of deaths and it accounts for 2.4%, that is 170000 of death per year in India. Compared to the data in 2000, death due to suicide has shown a rising trend over a 10 year period.

Million death study (MDS) estimates 3.7% of the total deaths in India are due to suicide that accounts for 187000 of the deaths which is higher than WHO estimates. MDS also concluded that after the age of 15 there is a cumulative lifetime risk of 1.7% for suicide and it is more prevalent in South India. They have also reported that half of the suicide death are due to poisoning.(11)The National Crime Bureau

statistics showed a 15.3% increase in suicides over the decade from 2004-2014. Tamil Nadu, Maharashtra and West Bengal belong to the states with highest rate of suicide. Tamil Nadu had the highest number of deaths in 2012 and second highest in 2014 contributing 12% of such deaths. (12)

SUICIDE DUE TO POISONING IN INDIA

According to the 2011 census in India, *"Injury, Poisoning & Certain Other Consequences of External Causes"* accounted for 7.4% of all medically certified deaths of which poisoning due to various substances contributed 17.2% of deaths in this category. These were the leading cause of mortality in the age group of 15-24 and 25-34 years accounting for 25.9% and 22% respectively. This may be just a tip of an iceberg as only 20% of deaths were medically certified.(13)

EPIDEMIOLOGY OF PESTICIDE POISONING WORLDWIDE

Pesticides are integral part to increased agricultural production and food sufficiency. However the increased availability and access to pesticides has also resulted in pesticide poisoning becoming a major public health problem in middle and low income countries. It is one of the common, cheap and easily available methods for deliberate self-harm. WHO initiative on preventing death due to pesticide poisoning reports a million cases of pesticide poisoning per year which accounts for 25000 of death per year (14). It is a major health problem in Asian countries such as India, China, Sri Lanka and Vietnam. Pesticide poisoning accounts for 60% of suicide mainly in rural areas of south East Asian countries.(1)

PESTICIDE POISONING INDIAN SCENARIO

The Million death study showed poisoning accounted for 49% of death due to suicide among men and 44% of the death due to suicide among women were due to poisoning It contributed around 92000 deaths among individuals who were older than 15 years. Of the poisoning, the majority of classifiable cases were to pesticide poisoning. The million death study also showed that poisoning was more in four southern states.(11)

Reasons for such high rate of suicides were multifactorial. Bankruptcy, illness, drug and alcohol abuse, love affairs and family disputes were some of the causes for suicide. (12) .

In the year 2014 National crime bureau reported that 10% of all poisoning in India are due to pesticide consumption. (12)

WHO-Initiatives to prevent Poisoning due to Pesticide (15)

WHO has made an initiative to reduce social burden due to pesticide poisoning. The Major Components of this initiative are- (1) To regulate the pesticide policies, (2) improve the medical care and mental health, (3) Better surveillance and poisoning monitoring, (4) training for safe handling, (5) Community interventions for safe storage of pesticides.

Legislative measures-Although WHO has recommened the ban of highly toxic pesticide compounds, these are widely available in many countries. Some countries

have enforced bans on WHO class I and II compounds that have reduced mortality and suicide rates due to poisoning in Sri Lanka, Jordan and in Samoa.

WHO has also recommended the use of closed containers and communal storage along with farmer's education as an active measure to reduce the poisoning rates.

EPIDEMIOLOGY OF POISONING IN CMC VELLORE

In the Christian Medical College and Hospital, Vellore OP poisoning accounted for 70% of all poisonings and 12% of all admissions to the medical intensive care unit. The overall case fatality ratio in published literature was 22.6%. However the case fatality of OP poisoning in our hospital is low (<5%).

The total number of pesticide poisonings at Christian Medical College in between 2009-11 were 621. The rank order of pesticide poisonings were: Organophosphates (54.4%), Pyrethroids(14.7%), Rodenticides(7.6%), and organochlorine(6.8%). 70% of Organophosphates were highly toxic class I and II pesticides, with less number of Class III. Annual admission rates of acute organophosphate poisoning are approximately 150 cases of acute organophosphate poisoning per year.(16)

THE GENERAL STRUCTURE OF ORGANOPHOSPHORUS

Organophosphorus compounds are esters of phosphoric or phosphonic acid. All organophosphates share some common chemical properties. Organophosphates contain a central phosphorus atom with a double bond to either sulphur or oxygen, R1 and R2 groups that are either ethyl or methyl in structure, and a group which is specific to the individual organophosphate(17). Toxicity of this compound was first

recognized in Germany By Lange and Kruger. These agents were subsequently used as nerve agents in warfare and also as pesticides.(18)

General Structure of organophosphorus compounds is shown in the figure - (Figure:1)(17)

R1, R2-aryl or alkyl group

R3-leaving group-halogen or aliphatic or aromatic or heterocyclic groups

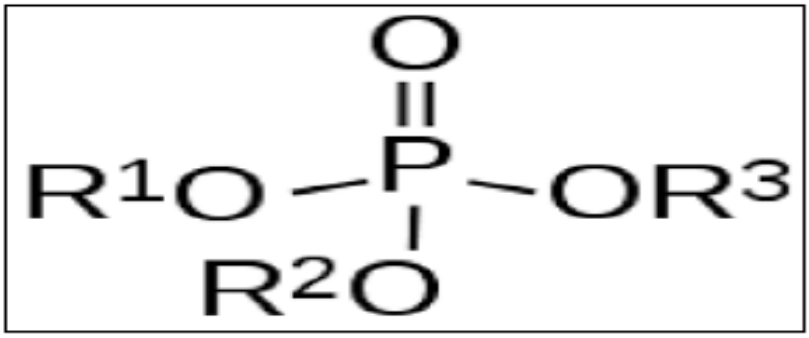


Figure 1-General structure of organophosphorus compounds

Organo phosphorus compounds have an alkyl group which can be either a methyl/ethyl/s-alkyl group. Studies have shown that severity of poisoning is related to type of compound also. Poisoning with dimethyl compounds have a worse outcome when compared to poisoning with ethyl compounds. This may be attributed to the fact that dimethyl compounds have a shorter half-life (half-life of dimethyl – 3 hours, diethyl – 30hours) and a lower capacity for regeneration with oximes(19).

CLASSIFICATION OF ORGANOPHOSPHORUS COMPOUNDS

1.WHO CLASSIFICATION BASED ON LETHALITY OF COMPOUND

This classification is based on the lethal dose (LD50) measures on rats both for oral as well as dermal exposure. Lethal dose (LD50) is amount of toxin (mg per kg of body weight) required for killing 50% of the population(20).

Table 1-Who classification based on LD 50

WHO CLASS	LD50 for the rat (mg/kg body weight)Oral	LD50 for the rat(mg/kg body weight) Dermal	Compounds
IA (Extremely hazardous)	**<5**	**<50**	**Parathion, Methyl-parathion**
IB(Highly hazardous)	**5-50**	**50-200**	**Dichlorvos, Dicrotofos, Monocrotofos, Triazophos**
II(Moderately hazardous)	**50-2000**	**200-2000**	**Chlorpyrifos, Diazinon, Fenthion, Quinalphos ,Profenofos,Dimethoate.**
III(Slightly hazardous)	**Over 2000**	**Over 2000**	**Methylchlorpyrifos, Malathion**
U-Unclassified (Unlikely to cause harm)	**5000 or higher**	**5000 or higher**	**-**

2. CLASSIFICATION BASED ON CHEMICAL STRUCTURE

Depending on the structure of R1 and R2 – as in chemical structure of organophosphorus molecule (21)

a. Diethyl – Chlorpyrifos, Quinalphos, Parathion

b. Dimethyl – Methyl-parathion, Monocrotophos, Fenthion, Dimethoate

c. S-Alkyl – Profenofos, Methamidophos

3.CLASSIFICATION BASED ON LIPID SOLUBILITY (21)

Compounds with higher lipid solubility have delayed onset of action and they are longer acting. Sudden release of compound from the tissue can result in sudden severe cholinergic crises and respiratory arrest.

Examples for the compounds with higher lipid solubility are- Fention, Dichlorfenthion, Parathion,and Chlorpyrifos

Among the organophosphorus compounds chlorpyrifos has more lipid solubility compared to other compounds.

4. CLASSIFICATION BASED ON WHETHER THEY ARE PRO POISON OR ACTIVE POISON (21)

a) Thion (pro-poison) – Parathion, Chlorpyrifos, Dimethoate – with sulphur atom attached to phosphate atom (P=S) and need conversion by CYP450 in gut wall or liver to active oxon (P=O) for clinical effects

b) Active oxon form – Profenofos, Dichlorvos – faster acting as they do not require activation .

MECHANISM OF ACTION OF ORGANOPHOSPHORUS COMPOUNDS

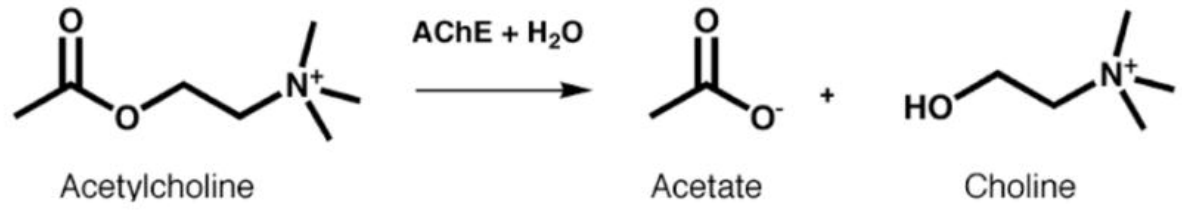
Clinical and toxic effects of Organophosphorus compounds are by its inhibitory action on acetylcholine esterase a neurotransmitter at synaptic nerve cleft.(2)

STRUCTURE AND FUNCTION OF ACETYL CHOLINE ESTERASE ENZYME

Acetyl cholinesterase (AChE) are serine hydrolases belonging to the family of type B carboxyl esterase and are specific cholinesterase present in the nervous system capable of hydrolyzing acetylcholine faster than other esterase into acetate and choline.(19)

Acetyl choline is a neurotransmitter in central, peripheral and autonomic nervous system. It is stored in the presynaptic nerve vesicles and is released into the synaptic nerve cleft during neurotransmission. Later its act on the receptors which are present on the postsynaptic membrane and leads to signal transmission.(Figure:5)(4)

Acetyl choline esterase terminates its action by hydrolysing the acetyl choline into acetate and choline. Choline later will be taken up into the vesicle and used for re-synthesis of the enzyme at presynaptic nerve terminals. Acetylated form of ACHE which is formed temporarily due to this action will react with a water molecule and an active enzyme is generated.(4)(Figure: 2)



Enzymatic hydrolysis of ACh by AChE.

Figure 2-Enzymatic hydrolysis of Acetyl choline

Without AChE, acetylcholine accumulates in the synaptic cleft leading to constant depolarization of the postsynaptic nerve causing the targeted muscle to remain contracted.(18)

STRUCTURE OF ACETYL CHOLINE ESTERASE(AChE)

Acetylcholine esterase enzyme has an ellipsoidal shape and it comprises of two active subunits anionic site(AS) and esteric site(ES)(4). (Figure: 7). Normally choline moiety of acetylcholine binds to the anionic site followed by hydrolytic action takes place in esteric site where the ester bond of acetylcholine is broken and leads to the formation of acetylated AChE and choline. Acetylated AChE later reacts with one molecule of water and forms acetate and free enzyme. Acetylation of the enzyme takes place in the serine –OH moiety of the esteric site and acetylated enzyme is an unstable intermediate hence rapidly react with water and releases acetylcholine esterase enzyme.(22)

Organophosphorus compounds have high affinity for acetyl choline esterase enzyme.

Organophospate poisoning inhibits hydrolases enzymes which includes both RBC and butryl choline esterases. However inhibition of butrylcholine esterase does not correlate with clinical profile of the poisoning.(19)

Before organophosphorus compounds binds to the acetylcholine enzyme activation of the compound takes place in liver via transformation at the double bond of the central phosphorus atom from sulphur to oxygen and convert organophosphorus into oxons which are more potent inhibitors of acetylcholine esterase enzyme.(19)

Like acetylcholine, organophosphorus compounds bind to the anionic site following which the esteric site is phosphorylated. This is analogous to the reaction which takes place in Acetyl choline hydrolysis where acetylation of the enzyme takes place. Phosphorylated enzyme is much stable than acetylated enzyme and hence hydrolysis of the same doesn't easily takes place.

Phosphorylated enzyme loses its ability to hydrolyse acetylcholine and hence this leads to accumulation of acetylcholine at the synaptic cleft which in turn will lead to the toxic clinical effect of organophosphorus poisoning.(23)

AGING OF CHOLINE ESTERASE ENZYMES

Progressive inhibition of cholinesterase by organophosphates results from phosphorylation of the active-site serine. Phosphorylated cholinesterases may undergo a dealkylation reaction of the organophosphorus moiety leading to "aged" enzyme, i.e. conversion of the inhibited enzyme into a non-reactive form. Aged AChE cannot be reactivated by oxime therapy.(24)

CLINICAL FEATURES OF ORGANOPHOSPHORUS POISONING

Clinical features and severity of organophosphate poisoning depends on the levels of acetylcholine esterase enzyme inhibition and it can manifest as immediate and delayed effects. Outcome of poisoning also depends on WHO class of compounds (25), Type of compounds (26), lipid solubility and biochemical characteristics.

Clinical syndrome can be classified into –

1. Acute (Minute to 24 hours) - Cholinergic crisis

2. Delayed (24 hours to 2 weeks) - Cholinergic crisis, Intermediate syndrome, Delayed organophosphorus related encephalopathy, Extra pyramidal symptoms, and delayed cerebellar effects.

3. Late(Beyond 2weeks)-OP induced delayed neuropathy.(27)

ACUTE CHOLINERGIC CRISIS

Acetyl choline acts via muscarinic and nicotinic receptors and stimulation of these will leads to nicotinic and muscarinic side effects and cholinergic crisis. Peripheral muscarinic receptor stimulation leads to commonly described " SLUDGE" symptoms components of which are- Salivation, Lacrimation, Urination, Diaphoresis, Gastrointestinal upset and Emesis' along with bronchorrhoea, bronchoconstriction , miosis and bradycardia. Peripheral nicotinic receptor stimulation at neuromuscular junction leads to muscle weakness, fasciculations and paralysis whereas at sympathetic system leads to tachycardia, hypertension, sweating and mydriasis. Both

nicotinic and muscarinic overstimulation at central nervous system leads to agitation,confusion,coma,seizures and respiratory failure.(19) Summary of various effects of acetylcholine on nicotinic and muscarinic receptors is described below.

Table 2-Clinical effects of OP by action on various receptors

Type of receptor	Receptor subtype	Action on	Manifestation
Nicotinic receptor stimulation	N1(Nm)	Neuromuscular junction	Weakness,fasciculations, cramps,paralysis
	N2(Nn)	Autonomic ganaglia Adrenal medulla	Tachycardia Hypertension
Muscarinic	M1-M5	Centralnervous system	Respiratorydepression,circulatory collapse,anxiety,resllessness,convulsions,insomnia,coma,ataxia,dysarthria, tremors
	M2	Heart	Bradycardia, hypotension
	M3,M2	pupils	Blurred vision, miosis
	M3,M2	Exocrine glands	Salivation,lacrimation,bronchorrhoea, rhinorrhoea,defecation
	M3,M2	Smooth muscle	Bronchospasm, abdominal pain and urinary incontinence

Death can be secondary to cholinergic crisis, cardio respiratory arrest, aspiration related to coma and seizures.(27)

INTERMEDIATE SYNDROME

Wadia et al first described the neuroparalysis of organophosphate poisoning classifying the syndrome into Type I-III paralysis. in 1974 (28), Senenyake etal were the first to use the term “intermediate syndrome” in patients with acute organophosphorus consumption which they defined as development of proximal muscle weakness developing 24-96 hours after the consumption of organophosphorus compound and after the acute cholinergic crisis has settled(29). They also studied the electromyography findings and postulated possible aetiology as a post synaptic defect for the cause of intermediate syndrome. Compounds that have found to be associated with intermediate syndrome in their study were fenthion, monocrotophos, metamidophos and dimethoate. As the syndrome occurred after the acute cholinergic syndrome but before organophosphate-induced delayed polyneuropathy, the syndrome was called 'intermediate syndrome'(29). From a study conducted in South India in 2005 by Vikram et al reported incidence of intermediate syndrome of 29.4%. Methylparathion, Monocrotophos, Phosphamidon, Quinalphos and Dimethoate were the identified compounds associated with IMS of which methylparathion poisoning was the commonest and contributed for 57.4% cases of IMS. Though most common manifestations were neck muscle weakness, proximal muscle weakness and respiratory muscle weakness there were cases with isolated cranial nerve palsies as well (30). From a study conducted from our institution the reported incidence of intermediate syndrome was 37.5%.Persistent and severe inhibition of acetylcholine esterase levels was noted among patients who developed intermediate syndrome. In this study the characteristic electromyographic findings in IMS were severe

progressive decrement response and decrement increment response in lower frequencies(31). Danadapani et al in 2002 also proposed organophosphorus induced oxidative muscle damage as the additional mechanism for development of IMS other than persistent choline esterase inhibition.(32)

The definition of Intermediate syndrome used in this study has been adapted from the definition proposed by Dr. John and Dr. Khan, from studies which were published in our institution.(33)

Intermediate syndrome is defined as development of proximal muscle weakness of MRC grading 3 or less along with extra-ocular, neck and respiratory muscles weakness developing after 72 hours of consumption of the organophosphorus compound, which may or may not require mechanical ventilation.

DELAYED ORGANOPHOSPHORUS RELATED ENCEPHALOPATHY (DOPE)

DOPE is a distinct clinical entity usually occurs after 4 days and characterized by reduction in Glasgow coma score, miotic non reactive pupils and an absent brain stem activity without evidence of vascular, infective or metabolic causes and can persist for a week or two. Electroencephalographic findings are suggestive of bihemispheric slow wave disturbances which is in favour of an encephalopathy.(34)Postulated mechanism for DOPE is attributed to delayed nicotinic effects in the central nervous system caused by slow release and redistribution of lipid soluble compounds.(27)

Other delayed effects recorded are extra pyramidal and cerebellar symptoms which resolves are self-limiting.(27)

LATE EFFECTS: Patients with OP poisoning can have delayed manifestations such as peripheral neuropathy, and neuropsychiatric manifestations in the form of behavioural changes. (27)

ASSESSEMENT OF SEVERITY OF POISONING (35)

Namba scale has been widely used for assessment of severity of poisoning in patients who present with acute organophosphorus poisoning.

Table 3- Namba grading for severity

Namba grade	Clinical presentation
Namba 1 or Latent	No clinical manifestations Severity assessed by measurement of serum cholinesterase levels which is inhibited by 10-50 %
Namba II or mild	The patient can walk but complains of dizziness, headache, numbness of extremities, nausea and vomiting, excessive sweating and salivation, tightness in chest, abdominal cramps or diarrhoea. Serum cholinesterase level is 20-50% of normal.
Namba III or Moderate	The patient cannot walk and there is generalised weakness, difficulty talking, muscular fasciculation, miosis. Cholinesterase level is 10-20% of normal
Namba IV or Severe	Unconsciousness, marked miosis and loss of pupil reflex to light, muscular fasciculation, flaccid paralysis, secretions from the mouth and nose, moist rales in the lungs, respiratory difficulty and cyanosis. Serum Cholinesterase levels are less than 10% of normal.

DIAGNOSIS OF ORGANOPHOSPHRUS POISONING

Organophosphorus poisoning in India is diagnosed based on clinical features as diagnostic tests are not widely available. Butrylcholine esterase levels (BChE) are used as a surrogate marker for diagnosis because of its ease of performance. Red cell choline esterase (RBC AChE) is more useful in assessing the clinical severity but is not routinely performed even at Christian Medical College, Vellore. RBC AChE has 3 isoforms of which AChE-S is seen on synapses and AChE-E is seen on RBC surfaces however they have similar mechanism of action. AChE-S (synaptic) and AChE-E (Erythrocyte) have similar enzymatic action however butrylcholine esterase is different in its mechanism of action. (36)

BUTRYLCHOLINESTERASE (BChE)

Butrylcholine esterase also called Pseudocholine esterase or Plasma choline esterase are enzymes which are found abundantly in Plasma. It is synthesized in the liver and regenerates faster than the acetyl choline esterase. Its mechanism of action is similar to ACHE however it rapidly hydrolyses the Butrylcholine than Acetyl choline as a substrate.(4) Its biological action is not known and is considered as a scavenging enzyme which detoxifies natural compounds.

BCHE levels are sensitive markers of OP exposure and are useful in diagnosis. However the level of inhibition does not correlate with severity of poisoning. Recovery following OP poisoning is around 7% of normal per day after eliminating the OP compound from the body.(37)

Butryl choline esterase has wide range of normal values ranging from 3000-7000U/L Plasma). Hence even minimal inhibitions can show values within normal limits. It can be affected by factors such as pregnancy, liver disease and drugs.

BCHE measurement can be affected by variation of commercial assays, substrate concentration of butrylcholine, and temperature.(19) From various studies it has been concluded that level of Pseudocholine esterase will help in diagnosis of the poisoning however it doesn't correlate with the clinical severity.(3)

RED CELL CHOLINESTERASE (RBC ACHE)

Acetylcholine esterase also called true choline esterase is present at the synapses in the nervous system and also on the RBC membrane. It plays a major role in hydrolysis of acetyl choline at synaptic junction and a minor role in cellular growth and adhesion.(4) Red cell regeneration following OP poisoning happens at a rate of less than 1% per day (19)and it takes an average of 82 days before the RBC-ACHE normalizes as it regenerate by erythropoiesis (37). However time taken for neuronal esterase recovery is unknown hence during recovery RBC ACHE is a less useful marker to assess the neuronal acetylcholine esterase activity.

A previous study conducted by Thiermann etal has reported good correlation between RBC acetylcholine levels and neuromuscular inhibition. They have reported that the level of RBC ACHE activity correlates with level of neurotransmission as evidenced by electromyographic findings. RBC ACHE activity of less than 10% which correlates with severe poisoning is associated with strongly impaired neuromuscular transmission which was reflected in the Electromyographic study as decrement phenomenon(6). Moderate inhibition (RBC AChE activity between 10-

30% and minimal inhibition (RBC ACHE value more than 30%) was associated with impaired and normal neuromuscular transmission respectively. From this study Thiermann et al concluded RBC-AChE should have comparable functional properties to synaptic AChE and therefore may be used as surrogate parameter, reflecting the AChE status at the synaptic site (6).

RBC AChE assays measure activity of AChE enzyme which is present on the red cell membrane. This enzyme is measured in whole blood in which butyrylcholinesterase activity has been blocked by an inhibitor. Assays are affected by concentration of substrate, pH,oxime concentration, room temperature, time taken for cooling and dilution.(19)

METHODS FOR DETECTION OF ACETYLCHOLINE ESTERASE LEVELS

LABORATORY METHODS

For measurement of acetylcholine esterase laboratory methods and rapid detection assays are available.

The most commonly used assays for routine Lab ChE testing are -

1. The delta pH method by Michel (1949).

2. The radioactive Ache method of Johnson and Russell (1975).

3. The kinetic assays pioneered by Ellman et al. (1961).

The Delta PH method (36)

It is a kinetic assay and in this method substrate acetylcholine bromide is treated with RBC or plasma. On further reaction substrate is hydrolysed to produce choline and acetic acid which causes a change in PH. The change in pH over time is expressed as delta change in pH per hour. This method is slow and not feasible in commercial laboratories.

The radioactive method(36)

This is a micro assay where radio labelled 3H-acetylchline is used as a substrate .Following reaction it produces hydronium iron which is measured in this assay. This is rapid and can be used for multiple ChE measurements; however it is expensive and has radioactive waste problems.

The Ellman Method(36)

The most commonly used assay is the Ellman method. This method uses the substrate acetylthiocholine and hydrolysis by enzymes leads to formation of thiocholine and acetate. Thiocholine reacts with 5, 5'-dithiobis-2-nitrobenzoic acid to produce an acid anion (5-thio-thionitrobenzoic acid) which produces yellow colour that can be measured with a spectrophotometer. The results are expressed as U/ml or μmol/min/mL). Compared with the above mentioned methods Ellman method is more sensitive in detecting mild level of enzyme inhibition.(36)

Other laboratory methods include WRAIR (Walter Reed Army institute of research) method developed by Gorden et al and analytic methods such as proteomics which was developed (36)clinical practice.(36)

Direct comparison of various methods are not available and conversion factors for delta PH ,Ellman and WRAIR methods have been suggested.(36)

FIELD TESTING KITS

Requirement of filed kits are- Accuracy and specificity, affordability, portability, capacity to measure and compare baseline enzyme levels and enzyme activity following exposure, ease of operability and stability at varying temperature and humidity.(36)

There are 2 commercially available field kits –

Tintometric field kit – This is the oldest kit which is available for the past 50 years. Current version is named as Lovibond CholinestraseAF267 test kit. Though it was found to be effective in determining the RBC AChE activity in severe poisoning, it is not effective in determining the baseline activity. Major drawback of this is it uses colour comparator method which is determined by the kit operator.(36)

Testmate™ ChE test Kit- It uses Ellman method to measure the RBC AChE and BChE values and it's a battery operated system which takes 4 minutes for the results. Enzyme values are determined using whole blood. Its results are expressed as U/ml for BChE, and U/gm Hb for AChE. Latest available model for the same is 400 Test-

mate™ ChE kit. This device is manufactured by EQM research Centre (Cincinnati, OH).(38)

Cholinesterase Rapid Test IVD /ChE check mobile - Bundeswehr institute of toxicology and pharmacology has developed a new device for field testing with the manufacturer securetec Detektions-Systeme AG, Brunn - thal. This is comparable with the Testmate kit approved by USFDA. This method uses modified Ellman method(8). Figure: 3 shows the Components of ChE check mobile kit which was used in this study.

Figure 3-Components of RBC AChE check mobile

Components of the kits are a ChE Measuring machine, Reagent kit which consists of a buffer containing cuvette, Reagent caps for determining AChE and BChE which are yellow for BChE and red for AChE measurements respectively, EDTA coated 10 microlitre capillaries and adsorbant tissue.

Part of the ChE Check mobile are- Touch pad with menu, data entry, instructions for use, temperature sensor, Rechargable battery, charger, internal storage and and a analysis unit for AChE, BCHE and haemoglobin based on photometric determination at a wavelength of 470nm.

The measurement of enzyme activity in the whole blood is by using the modified Ellman method which is different from the original Ellman by the use of BChE Inhibitor Ethopropazine, more temperature stability, and use of a different wavelength of 436nm. 436 nm reduces the background haemoglobin absorption and by using selective BChE Inhibitor, Enzymes assays can be done in whole blood and unnecessary centrifugation can be avoided. Validation studies for these methods have been published.

Testmate and check mobile uses similar mechanism that is modified Ellman method for the detection of cholinesterase with the use of inhibiting agents and different substrate for depends on AChE/BChE detection. Manufacturer has claimed good correlation when compared with modified Ellman method with r value of 0.93 for ACHE and 0.96 for BCHE on 10 samples.(8)

VALIDATION STUDIES

Validation studies are available for the testmate however for checkmobile is not validated in clinical scenario.

Comparative analysis done by Haigh et al on 47 healthy volunteers where they compared WRAIR assay with testmate, microellman method and delta PH method.

There was good correlation for RBC AChE between WRAIR with alternate method with r2 = 0.83-0.93. However for BCHE correlation was less on comparing alternate methods r2 values 0.5 - 0.6.(39)

Another validation study done in Srilanka by Rajapakse et al on acute OP poisoning patients showed good correlation between lab values and Testmate in measuring RBC AChE. For AChE and BChE Spearman's correlation were 0.87 and 0.76 respectively.(40)

CLINICAL IMPORTANCE OF POINT OF CARE TESTING (POC)

From various studies it was found that point of care testing is an invaluable tool which increases diagnostic accuracy, and clinicians confidence in decision making in rural settings (41). Blattner etal also found that POC testing in addition to increasing the diagnostic accuracy it causes reduce transfer rates an decreases financial burden.(42)

Clinical importance of point of care testing was evaluated in rural Sri Lanka among 23 clinicians In this study they have assessed the knowledge, attitude and practice on acetylcholine esterase point of care testing and they have found clinicians with minimal experience or no experience found these test to be 100% useful in treating the patients(43)

Point of care testing of AChE has not been studied in Indian setting.

Most of our government hospitals and rural hospitals doest have facility for determination of BuCHE or Acetylcholine esterase assay.In our study we are aimed at validating the rapid check mobile for detection of RBC AChE and BChE determination so that it can be made available in rural setting for management of patients with Organophosphorus poisoning. That will help us making important clinical decision such as discharge, duration of hospital stay and need for ICU/Ventilatory support. This study also intends to assess the role of serial BChE and Ache in terms of prognostication, including predicting the need for mechanical ventilation and development of intermediate syndrome.

JUSTIFICATION

From the above literature it is clear that organophosphorus poisoning is one of the common methods of deliberate self harm in India and low income countries as most of the population are dependenet on agriculture for their daily bread. More than that in low income countries, it is an easily available, cheap method of suicide among low and middle socioeconomic group .Most of the patient present to emergency department with history of an unknown poisoning and hence availability of rapid detection method will help in early detection of poisoning along with early initiation of specific therapy. Hence this study is planned to validate RBC Check mobile which was developed in Germany in Indian population so that it can be made available in resource poor setting for early detection of OP poisoning. Studying the correlation of RBC acetyl choline esterase in predicting the clinical outcome will help in decision making in clinical setting. Thus the proposed research is a study to assess the utility of RBC rapid check mobile in detecting the acetyl choline esterase concentration in the blood of patients as a diagnostic test for acute Organophosphorus poisoning.

METHODOLOGY

SAMPLE AND SETTING

This study was conducted over a period of 1 year from April 2015 to March 2016. All patients presented to the adult emergency department of CMC Hospital, Vellore with acute organophosphorus poisoning were assessed prior to recruitment into the study.

The principal investigator was contacted as soon as patients with organophosphorus poisoning came to emergency department. The procedure of the study was explained in detail to the patient and the relatives. A patient information sheet in their native language was also provided. Those who fulfilled the inclusion criteria and those who gave consent for participating in the study were recruited. Consent was obtained in their native language and in cases where patients were not fully conscious, consent was obtained from the accompanying patient's attendant and a re-consent was taken from the patient following recovery. As we saw cases of organophosphorus poisoning among those who were aged less than 18 years and pregnant women the protocol was amended to include pregnant women and those who were between the age of 15-18 years. For participants aged 15-18 years consent was taken from the guardian and assent was taken from the participant.

STUDY DESIGN

This study was a prospective study which had 2 parts:

(1) Validation study -To evaluate the diagnostic test accuracy of RBC point of care testing and

(2) A Clinical cohort study -To assess the temporal profile among patients with organophosphorus poisoning.

CASE ENROLLMENT

Consecutive patients admitted to the emergency department or general medicine wards with a diagnosis of organophosphorus poisoning were recruited into the study till sample size was achieved. Cases for the study were identified based on the following criteria-

1. Patients who present with history of pesticide poisoning with an identified OP compound.
2. Patients who present with history of pesticide poisoning with typical toxidrome of organophosphate poisoning, and low BChE levels (<3000 U/L).
3. Patients who present without a history of pesticide poisoning but with typical toxidrome of OP poisoning and low BChE levels (<3000 U/L).

Inclusion and exclusion criteria used for case recruitment were-

INCLUSION CRITERIA

1. All patients above age 15 years who come to adult Accident and Emergency at Christian Medical College with acute OP poisoning.

2. Patients who present < 24 hours after ingestion

EXCLUSION CRITERIA

1. OPP patient who were admitted into another hospital and presented after 24 hours.
2. OPP patients with time since consumption > 24 hours
3. Patients who are known to have hemoglobinopathies
4. Patients who received anti malarial treatment 2 weeks prior to consumption.

STUDY PROCEDURE

VALIDATION STUDY

A total of 10 consecutive non-OP pesticide poisoning and a total of 40 normal ICU and department of medicine staff (20 men and 20 women) from the hospital staff of different ages were planned to be taken as normal controls. Based on the sample size 10 patients with OPP poisoning were planned to be recruited in the study.

All normal controls and non OP poisoning controls had one measurement of rapid RBC check mobile with simultaneous lab measurement of serum BChE and RBC AChE.

All OPP cases had RBC AChE and BChE with rapid check mobile at admission, 12 hours after admission and daily for the first 7 days or till discharge with simultaneous measurement of serum BUCHE and RBC ACHE in blood samples.

Validation of whole blood Butrylcholine esterase levels using rapid check mobile was not planned due to the following reason. The whole blood BChE measurement by the check mobile machine does not have correction for haemoglobin concentration. Hence it cannot be validated against laboratory serum BChE which corrects for haemoglobin concentration. However temporal profile of BChE by rapid check mobile was used to document recovery. Hence rapid check mobile BChE was performed along with RBC AChE at the same time points at admission, 12 hour and daily upto the point of discharge or up to 7 days.

SAMPLE SIZE FOR VALIDATION STUDY

It is aimed to have 80 percent correlation between the machine and the current method of estimating choline esterase levels. In order to test that it was greater than 50%(null hypothesis value)with alpha and beta errors 5 and 20 percent respectively with 2 sided test sample size calculated for validation study was 30 samples.

Validation study was planned to conduct on 130 samples which included

1. Normal controls-40 normal objects (20 male and 20 female)

2. Non OP pesticide poison -10 cases (Admission value)

3. Organophosphate poisoning patients (OPP patients)-10 cases.

--5 Cases of Mild /Moderate poisoning (by Namba scale) and 5 cases of severe poisoning

--Values at admission, 12 hours later and daily at a fixed time of the day upto discharge or upto 7 days. (Total 80 Samples).

Only after standardisation of the machine, the Rapid check mobile was used daily to correlate the temporal profile of RBC Ache and BChE to clinical profile.

CLINICAL COHORT STUDY- MEASUREMENTS – DATA AND SAMPLE COLLECTION

A consecutive sampling strategy was employed for this study, wherein all adult patients presenting to the Emergency department at CMC Vellore with suspected acute OP poisoning were considered for enrollment in the study. A total of 59 cases of OP poisoning were recruited into the study. After obtaining consent admission clinical data was assessed and documented in patient's performa.

Data collection was done by the principal investigator at patient presentation to CMC, Vellore followed by serial assessment 12 hours later and subsequently once daily between 6-8 PM till discharge. The clinical data was documented in the patient proforma (Annexure 3) by the principal investigator. The following details were noted specifically

1. **Compound characteristics** – class, quantity and combination with pyrethroids

2. Time to presentation to CMC, Vellore

3. **Treatment elsewhere** – Gastric lavage, Atropine and Pralidoxime received including the doses

4. Clinical features at presentation-Cholinegic crisis

5. Treatment received in CMC- gastric lavage, duration of atropine, total dose of atropine

4. **Severity at presentation** (by Namba scale)

5. **Outcomes** – Dose of atropine required and duration, Mechanical ventilation and duration, Intermediate syndrome and duration, Need for ICU admission and duration

LABORATORY ASSESSMENT

As a part of the study experts from Germany who had developed the instrument trained respiratory technician regarding the correct use of RBC check mobile to measure the baseline BChE and AChE levels using the check mobile. The RBC check mobile measurements were performed at admission in the ward or ICU, 12 hours after admission and daily on 7 consecutive days at 12 noon. If the level of the enzymes were low, the estimations were extended till clinical improvement.

Samples for cholinesterase test were collected at admission not later than 24 hours after OP consumption followed by repeat sample 12 hours later and subsequently once daily at 12 PM for 5 days from poison consumption. The protocol for data and sample collection has been detailed in Annexure 4.

SAMPLE SIZE FOR CLINICAL COHORT STUDY

There were no studies that have correlated cholinesterase studies to the clinical profile. Hence it was not possible to calculate the sample size for the clinical study. After discussion we decided on sample size of 50 for a pilot study. We assumed to have 25 cases with mild/moderate poisoning and 25 cases with severe poisoning among the 50 patients.We also assumed 15 patients to develop intermediate syndrome and 30 patients to require mechanical ventilation. We expected this sample to have a distribution of 35 cases of WHO Class I and 15 with WHO Class II and III OP poisons and 25 cases of di-methyl and 25 cases of di-ethyl OP compound poisoning.

Detailed algorithm of the study is described in the annexure.

DETERMINATION OF CHOLINESTERASE ENZYME LEVELS

All patients had the following investigations done on the samples collected as mentioned above:

1. RBC-AChE activity Using RBC AChE rapid check mobile and standard laboratory method
2. Plasma BChE activity using standard laboratory methods and Blood BChE activity using RBC AChE rapid check mobile.

Reference standard used in our lab is measurement of plasma BChE and RBC-AChE. We use modified Ellman assay for detection of cholinesterase levels in our lab. After recruitment into the study, venous blood samples were collected from the patient and 2 ml of blood was added to a purple EDTA tube. For RBC AChE analysis, from the collected blood samples, 200 microlitres of blood was measured using a pipette and diluted (20 times) in 4 ml of cold normal saline (at 40 degree C) to prevent hemolysis, which was then well mixed and stored in -20 degree Celsius in the refrigerator within 5 minutes. The blood samples were sent to laboratory in batches at the end of 7 days for each patient. The remaining sample in the EDTA tube was then sent for BChE analysis immediately to the lab. Both the assays used the same principle measured through an automated assay. This method measures hydrolysis of the substrate acetylthiocholine (ATCh) by either BChE or AChE to yield acetate and thiocholine. The latter product reacts with 5, 5‘-dithiobis-2-nitrobenzoic acid to produce a yellow-coloured acid anion (5-thio-thionitrobenzoic acid) that can be measured with a spectrophotometer. The results were expressed as micromoles per minute per millilitre (μmol/min/mL) and adjusted for haemoglobin absorbance.

For the measurement of the Machine AChE and BChE 10 Microlitre of blood in 2 capillary tubes which were EDTA coated were drawn from the venous sample and was used for further detection of enzyme levels. The Standard Operating procedure used for both laboratory and point of care testing is mentioned in detail in annexure.

DATA ANALYSIS AND STATISTICAL METHODS

Data was collected in data extraction form (appendix) and was entered using epidata software and was analysed using SPSS software

STATISTICAL METHODS FOR VALIDATION STUDY

Choline esterase levels were measured using the current method and the machine. The scatter plot of these two readings were done to study the trend and correlation between the two methods. Mean difference plot were done to explore deviations or estimate the variations due to high or low values of choline esterase.

Intra class correlation co efficient was done to estimate the correlation between two samples.

STATISTICAL METHODS FOR CLINICAL COHORT STUDY

Correlation of admission BChE and AChE and association to severity of poisoning, GCS at admission, need for mechanical ventilation and development of intermediate syndrome were done by auto correlation using time series analysis of these reading over 7 days/till discharge.

Two point variables were compared using Chi-square test and continuous variable were correlated based on t test. The correlation of choline esterase level in intermediate syndrome has been presented as relative risk, by having acetylcholine esterase value less than or more than 95 percent of confidence interval. As AChE

levels were measured over 7 days as longitudinal data analysis, GEE method was used to analyze serial data. The effect of other confounders was adjusted using GEE method.

FUNDING AND APPROVAL

SOURCE OF FUNDING

A FLUID research grant was approved from the institution for the purpose of this study. The funds were used for the cholinesterase status assays.

INSTITUTIONAL RESEARCH BOARD APPROVAL AND ETHICAL CONSIDERATIONS

The research proposal for this study was discussed by the Institutional Review Board in 2014 and approval was obtained [IRB Min. No. 9203 dated 14.12.2014]. There were no ethical issues related to this study. Institutional review board approval was obtained for the procedures. Amendment to include pregnant women children aged between 15-18 years was approved in November 2015 (Ref.IRB.A3-10/11/2015).

RESULTS

Part 1- Clinical profile of OP poisoning

Part 2- Clinical Validation study of Point of care AChE estimation

Part 3- Clinical correlation study of Point of care AChE estimation

PART 1- CLINICAL PROFILE OF OP POISONING

DEMOGRAPHIC CHARACTERISTICS (Table 4)

59 patients > 15 years of age, admitted in emergency department of Christian Medical College, Vellore and who gave written consent were included in this study. The mean age (± SD) of patients was 30.56 ±12.98 and 76.3% belonged to age group of 15-35 years. There were total 32 men (54.2%) and 27 women (45.8%). 38 were married. One lady was pregnant presenting in second trimester.

37.3% of patients were educated. 62.7% were uneducated and majority doing unskilled labour.

Table 4-Demographic details

Variables	Numbers (N-59)	Percentage%
Age-		
<=25	29	49.2
26-35	16	27.1
36-45	5	8.5
>45	9	15.3
Average Age (Mean±SD)	30.56 ±12.98	
Sex		
Male	32	54.2
Female	27	45.8
Pregnant	1	3.7
Marital status		
Single	21	64.4
Married	38	33.9
Educational status		
Educated	22	37.3
Uneducated	37	62.7

ORGANOPHOSPHORUS COMPOUND CHARACTERISTICS (Table 5)

53(89.8%) of the 59 cases presented with an identified compound. In 66%of cases the compound was identified by the bottle of the pesticide which was brought by the patient's relatives, 24.5% by the leaflet which was brought by the relatives (24.5%) and in 9.4% by the compound name was given by the relatives (9.4%). In 6 cases the diagnosis of OP poisoning was based on clinical criteria of diagnosis (history of pesticide ingestion or typical toxidrome with low BChE levels).

The most commonly consumed compounds in rank order were Triazophos (15.25%), chlorpyrifos (15.25%), Profenofos (13.6%) and Monocrotophos (11.86%). Other commonly consumed pesticides were Phorate (6), Dimethoate (5), Dichlorvos (3), Quinalphos (2), and Malathion (2). 34% of the compounds were dimethyl OP compounds and 49.1% diethyl OP compounds and 15.1% S-alkyl compounds.

27.3% of cases consumed combinations of pyrethroids with OP most which were Cypermethrin combinations.

Table 5-Compound characteristics

Variables	Numbers (Total N-59)	Percentage(%of total patients)
Number of people with identified OP compound	53	89.8
Compound identification method		
Name given by patient	5	9.4
Leaflet brought	13	24.5
Bottle brought	35	66
Compounds		
Monocrotophos	7	11.86
Dimethoate	5	8.47
Profenofos	8	13.6
Triazophos	9	15.25
Chlorpyrifos	9	15.25
Phorate	6	10.17
Quinalphos	2	3.389
Dichlorvos	3	5.084
Malathion	2	3.389
Ethion,	1	1.694
Methylparathion	1	1.694
Type of compound		
Diethyl	26	49.1
Dimethyl	18	34
s-Alkyl	8	15.1
Others	1	1.9
Pyrethroid combination with OP	16	27.1
Pyrethroid type		
Cyhalothrin	1	1.7
Cypermethrin	11	18.6
Deltamethrin	4	6.8

DETAILS OF TREATMENT RECIEVED OUTSIDE (Table 6)

79.7% of patients were treated in another hospital prior to presentation to CMC. The average time taken for the first medical contact was 1.82±1.83 hours. The average time to presentation to CMC was 6 hours. Some patients were taken to more than one hospital prior to presentation. The majority had received treatment prior to presentation that included: gastric lavage (71.2%), induced emesis (15.3%), atropine (51.3%) and PAM (23.7%) and intubation (11.9%). Atropine dose received prior to presentation ranged from 2-40 ampoules (18 patients) and dose of PAM (oxime) received outside ranged from 1 to 5gm.

Table 6-Treatment received outside

Variables	Frequency
Average time taken for first medical contact	1.82±1.83
Treatment received outside	47(79.7)
No:patients who had skin decontamination outside	0(0)
No:patients who had induced vomiting outside	9(15.3)
No:patients who had gastric lavage	42(71.2)
No:patients who had received atropine outside	35(51.3)
No:patients who had received PAM outside	14 (23.7)
No: patients who were intubated outside	7(11.9)

TOXIDROME AT PRESENTATION (Table 7)

The majority of patients presented with the typical toxidrome: salivation (61%), lacrimation (22%), diaphoresis (20.3%), urination (15.3%), defaecation (10.2%), vomiting, (72.1%), breathlessness (33.9%) and altered sensorium (55.9%). 3 patients were asymptomatic at presentation

Table 7-Toxidrome at presentation

Toxidrome present	Number of patients (%)
Salivation	36(61)
Lacrimation	13(22)
Diaphoresis	12(20.3)
Urination	9 (15.3)
Defecation	6 (10.2)
Vomiting	43 (72.1)
Seizures	7 (11.9)
Breathlessness	20 33.9)
Altered sensorium	33 (55.9)
Abdominal pain	3(5.1)
Drowsiness	23 (39)
Giddiness	2(3.4)
Agitation	15 (25.4)
Asymptomatic	3 (5.1)
Frothing	13(22)
Bleeding	0(0)
Fever	4(6.8)

SIGNS AT PRESENTATION (Table 8)

27.1% of patients presented with GCS score < 10 and 42.4% had normal GCS at presentation. 35.6% patients had pin point pupils. Mean heart rate was 108.02±-22.85 and blood pressure was 116.44(±21.92)/74.07(±10.52). 7 patients had already been intubated at presentation and 3 were gasping at presentation. Single breath count (SBC) and neck holding time (NHT) could not be assessed among 31 patients at presentation. Average SBC and NHT were 21.6±9.41 and 24.97±21.90 seconds respectively.

Table 8-Signs at presentation

Clinical signs	Number of patients with % (or Mean ± S.D)
GCS, n (%)	
15/15	25(42.4)
10-14	18 (30.5)
<10/15	16 (27.1)
Pupil size, n (%)	
Pinpoint	21 (35.6)
Dilated	4 (6.8)
Normal (2-5mm)	34 (57.6)
Heart rate, mean (± S.D)	108.02±22.85
Blood pressure, mean (± S.D)	116.44(±21.92)/74.07(±10.52)
Respiratory rate, mean (± S.D)	
Mean RR	24.02±7.25
Intubated	7
Gasping	3
O2 saturation, mean (± S.D)	93.32±-9.23
Blood sugar, mean (± S.D)	173.73±60.50
Crepitations	27 (45.8)
Paradoxical breathing	13(22)
Abdominal tenderness	0
Fasciculations present, n(%)	13 (22)
Single breath count, mean (± S.D)	
Mean	21.62±9.41
Could not be assessed	31
Neck holding time , mean (± S.D)	
Mean	24.97±21.90
Could not be assessed	31

SEVERITY OF POISONING (Table 9)

The majority of patients were severe (49.2%) or moderate poisoning (20.3%) based on Namba scale. 25.4% were mild and 5.1% latent poisoning (asymptomatic).

Table 9-Severity of poisoning

Severity (Namba scale), n (%)	**Number (%)**
Latent	**3 (5.1%)**
Mild	**15(25.4%)**
Moderate	**12(20.3%)**
Severe	**29(49.2 %)**

DESCRIPTION OF TREATMENT (Table 10)

84.7% received gastric lavage at admission to CMC emergency department. Those who presented late did not receive gastric lavage. 96.6% required atropine infusion with an average dose of 245 mg and average duration of atropine infusion was 4 days. 20.3% developed atropine delirium. 78% of patients received activated charcoal at admission.

Table 10-Treatment received in CMC

VARIABLES	FREQUENCY-N (%)
Average time taken to reach CMC	6±5.16
Atropine **Required, n (%)** **Dose, mean (± S.D.)** **Duration, mean (± S.D.)**	 57 (96.6) 245.12±332.53 4.37 (± 4.07) days
Atropine delirium	12 (20.3)
Gastric lavage	50(84.7)
Activated charcoal	46(78)
Ionotropic support	5 (8.5)

CLINICAL OUTCOME (Table 11)

28.8% patients developed intermediate syndrome and average duration of intermediate syndrome was 8 days. 59.3 % patients required both ICU admission and mechanical ventilation of which 11.9% required tracheostomy. The most common indications for mechanical ventilation were low sensorium and respiratory muscle weakness. The mean duration of ventilation was 7.2 days and mean duration of ICU stay was 8.5

days. 30.5% patients developed nosocomial infections which were ventilator associated pneumonia and bacteremia.

Two patients developed delayed organophosphorus related encephalopathy and 3 patients died during the hospital stay. Of the 59 cases, 53 patients recovered completely (89.83%), 3 patients were discharged against medical advice (5.09%) and 3 patients died during hospitalisation (5.09%). One patient died during the cholinergic crisis and two patients died of hospital acquired infections.

Table 11-Main Clinical outcome

Clinical outcome	**Number of patients with % (or Mean ± S.D)**
Intermediate syndrome, n (%)	17(28.8)
Duration	
<=5	9(15.3)
>5	8(13.6)
Average duration (Mean+/SD)	8.18±7.04
ICU admission, n (%)	35(59.3)
ICU duration	8.47±6.18
<5	14 (23.7)
6-10	12(20.3)
>10	9 (15.3)
Average duration (Mean±SD)	8.47±6.18
Mechanical ventilation, n (%)	35 (59.3)
Indication for mechanical ventilation, n (%)	
Low sensorium	
Respiratory muscle weakness	19 (32.2)
Type 1 respiratory failure	12(20.3)
	4(6.8)

Duration, (n (%)	
<5	18(30.5)
6-10	9(15.3)
>10	8(13.6)
Average duration (Mean±SD)	7.24±6.17
Tracheostomy required, n (%)	7(11.9)
Infective complications present, n (%)	18 (30.5)
Ventilator Associated Pneumonia	12(28.8)
Bacteraemia	3 (5.1)
DOPE* n (%)	2 (3.4)
Atropine delirium, n (%)	12(20.3)
Cardiac arrest, n (%)	3 (5.1)
Respiratory arrest, n (%)	4(6.8)
Average duration of hospital stay (Mean±SD)	9±6.08
Outcome, n (%)	
Alive	53(89.83)
Discharged against medical advice	3(5.08)
Death	3(5.08)

DOPE- Delayed Organophosphate induced encephalopathy

PART 2- CLINICAL VALIDATION STUDY OF AChE CHECK MOBILE

RESULTS FOR THE VALIDATION STUDY

The primary objective of this study was the validation of RBC AChE check mobile for measurement of RBC AChE and BChE against standard laboratory methods. For the validation of RBC AChE check mobile AChE and BChE levels were measured for 10 normal controls, 10 non OP pesticide poisoning and for 30 patients with OP poisoning along with simultaneous measurement of laboratory AChE and BChE.

Statistical correlation of AChE and BChE values between laboratory and machine methods were done by the following statistical methods: *bland altman analysis with intraclass correlation coefficient (ICC)* and also using the *scattered plot methods*.

The following comparisons were made between AChE check mobile (Machine AChE and BChE) and laboratory values:

1-Overall correlation: All subjects including OP poisoning, non OP poisoning at admission and normal controls

2- Correlation in patients groups:: OP poisoning patients, non OP poisoning at admission and normal controls separately.

3-Correlation of OP poisoning at admission and on different days of poisoning: OP poisoning at admission and values on the first 7 days of hospitalisation.

4- Correlation according to severity of OP poisoning (Namba scale) and type of OP compounds

1.OVERALL CORRELATION OF RBC AChE and BChE AMONG ALL SUBJECTS (OP poisoning, non OP poisoning, and normal controls)-(Figure 4)

Overall correlation between AChE Machine values and AChE laboratory values was performed using samples from all subjecting including OP poisoning patients (admission value), normal controls, and non OP pesticide poisoning. Using the scattered plot method a very good positive correlation was obtained between AChE Machine values and AChE Lab values with a correlation coefficient of 0.743($p<0.001$).

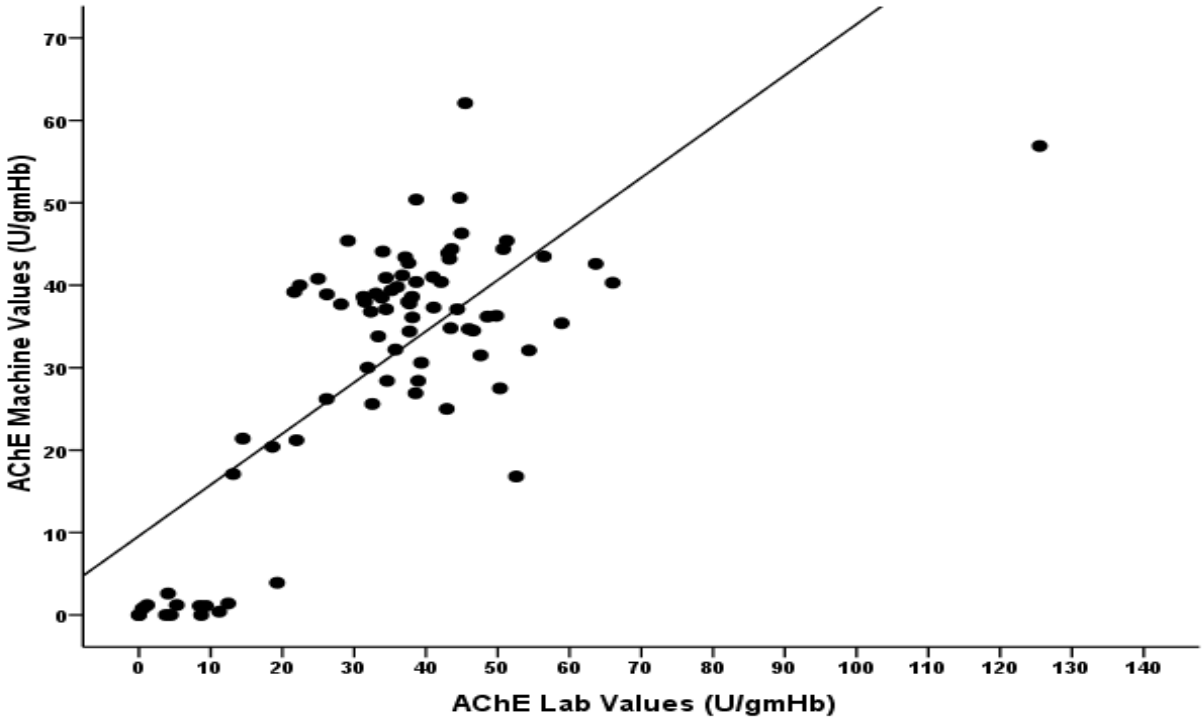


Figure 4-Overall correlation between lab AChE and Machine AChE among patients and controls

Figure 5 shows good correlation between BChE Machine values and BChE Lab values with the correlation coefficient of 0.875 ($p<0.001$) among normal and non-OP poisoning controls and patients with Organophosphorus poisoning.

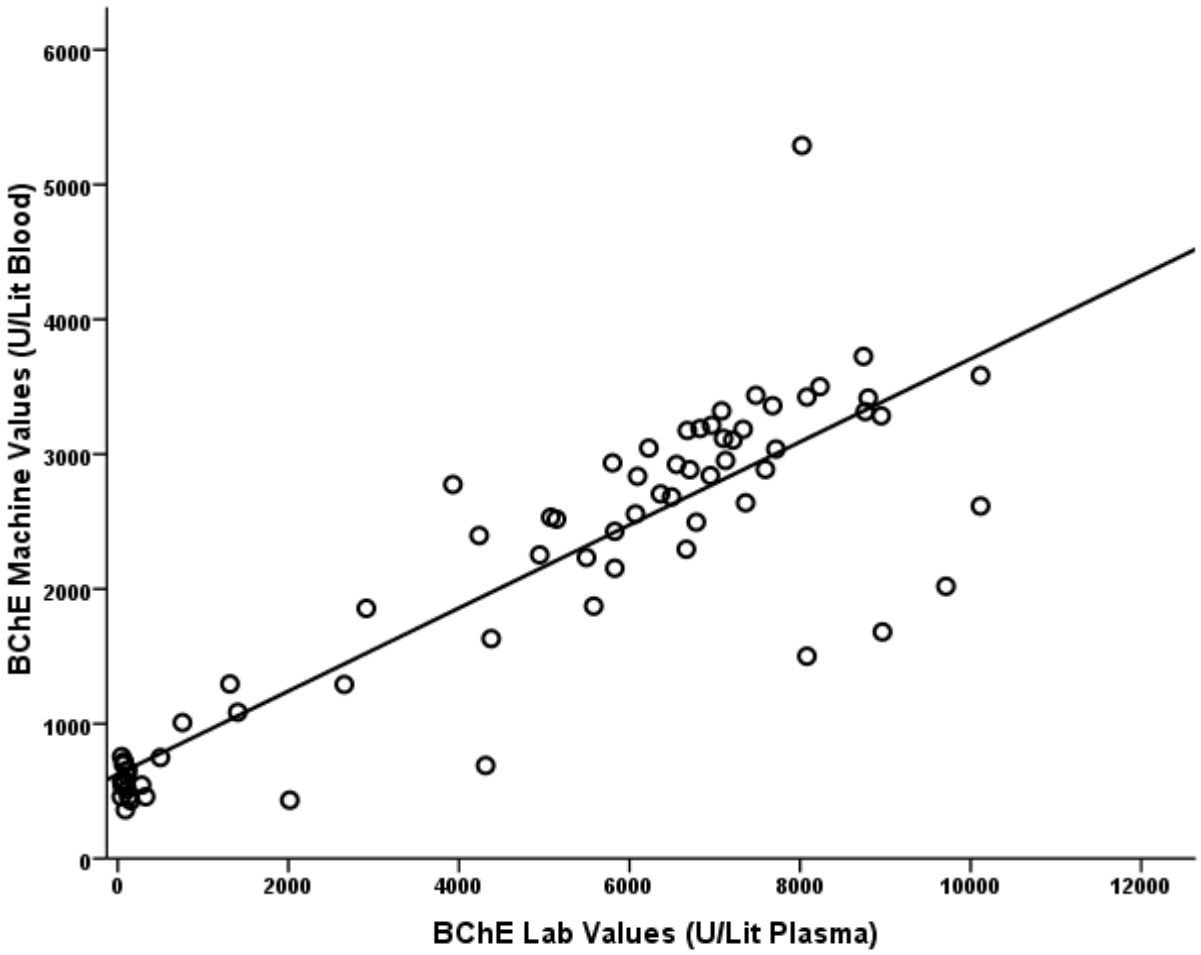


Figure 5-Overall Correlation between BChE Machine values and BChE Lab values for patients and controls.

2.1OVERALL CORRELATION OF AChE AMONG ACUTE OP POISONING (Figure 6 and 7)

There was a good agreement between two methods as shown below using Bland Altman plot and scattered plot analysis.

Figure 6 shows an overall correlation of AChE between 2 methods among patients with OP poisoning using Bland altman analysis. The Intra class correlation for AChE Machine values and AChE lab value is 0.858 (95% CI: 0.807 – 0.895) (p value <0.001). This ICC of 0.86 indicates that there is a good agreement between the two methods.

Bias = -10.03, **SD of Bias** = 16.498**,Limits of Agreement** = (-42.371, 22.302)

Precision (Upper limit-bias) = 22.302– (-10.03) = 33.332

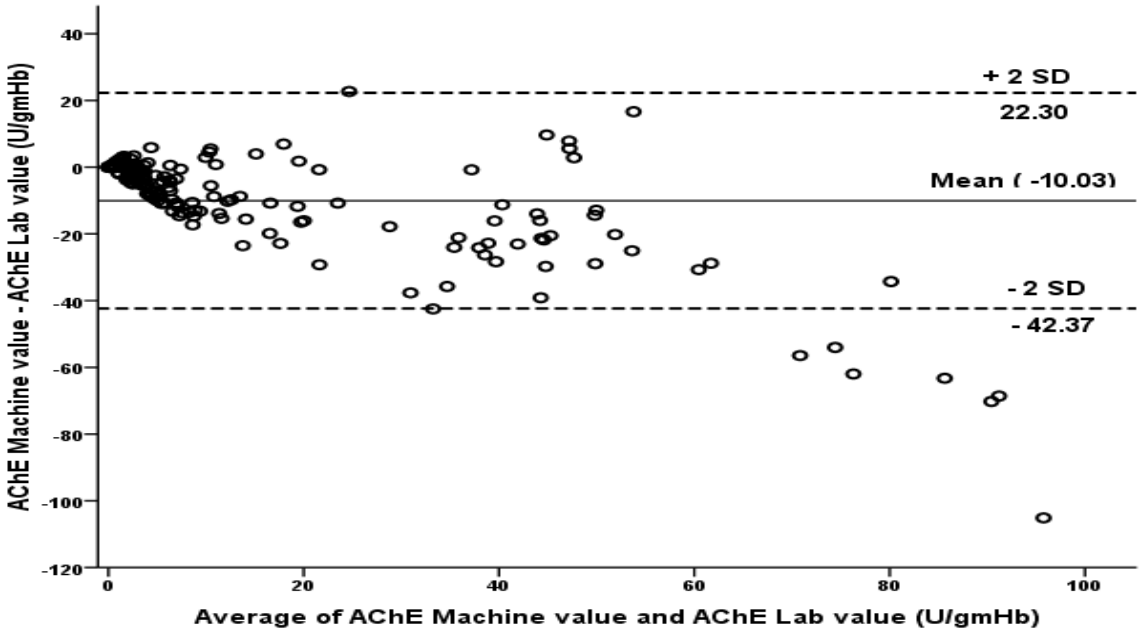


Figure 6-Bland Altman plot for AChE machine values and AChE lab values for 7 days.

Scattered plot analysis

Figure 15 showing Overall Correlation of AChE among 30 patients with OP poisoning over 7 days by scattered plot method and there is good correlation between AChE Machine values and AChE Lab values with a correlation coefficient of 0.868 ($p<0.001$).

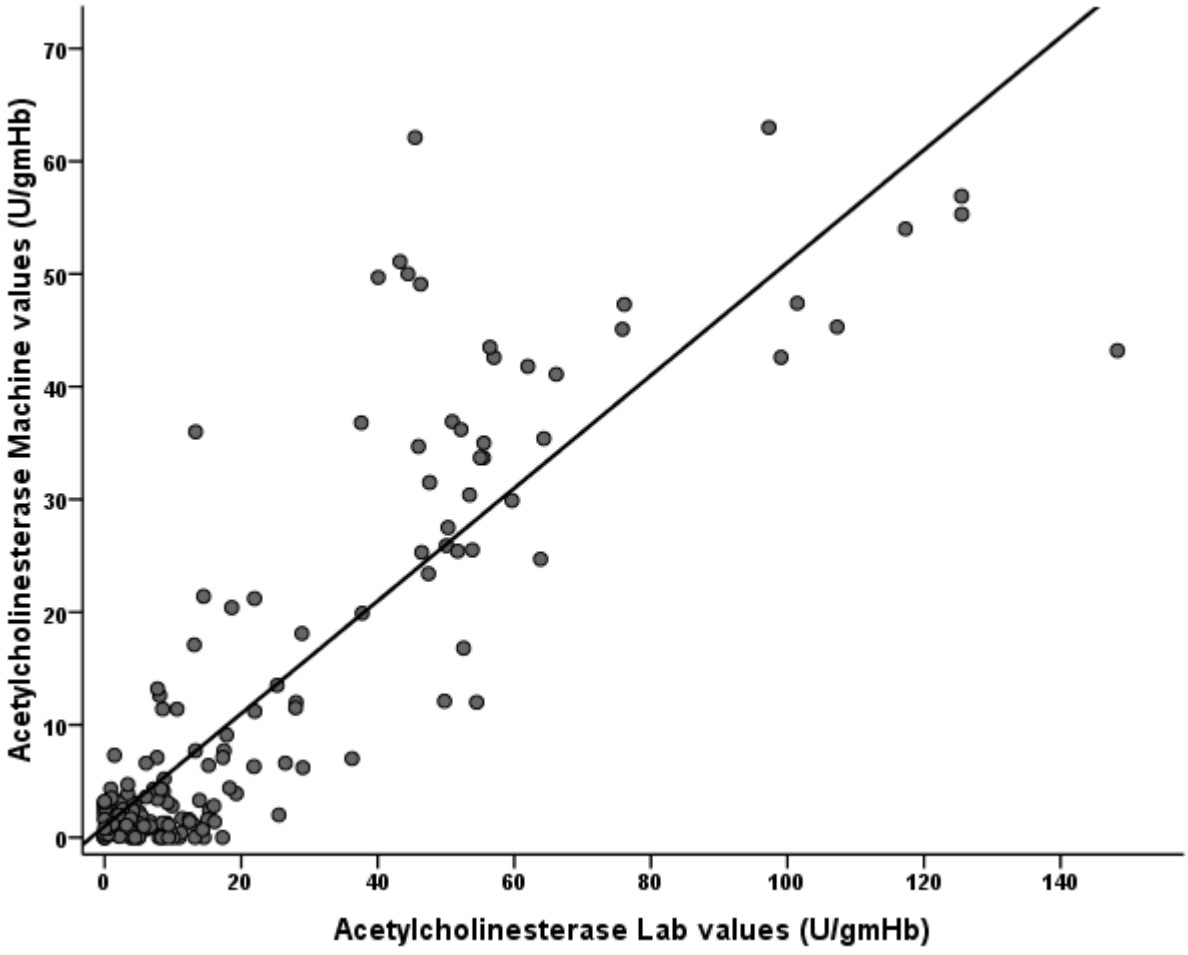


Figure 7-Overall Correlation of AChE among 30 patients with OP poisoning over 7 days by scattered plot method

2.2OVERALL CORRELATION OF BChE AMONG PATIENTS WITH ACUTE OP POISONING (Figure 8)

Overall correlation of BChE between 2 methods among patients with OP poisoning was not done by Bland altman plot analysis as lab value was expressed in U/L plasma and the machine Values were expressed in U/L of blood.

Figure 8 shows Correlation between BChE Machine value and BChE Lab values based on scattered plot method and the correlation coefficient was 0.927 ($p<0.001$) which suggest very good agreement between two methods in detecting BChE levels.

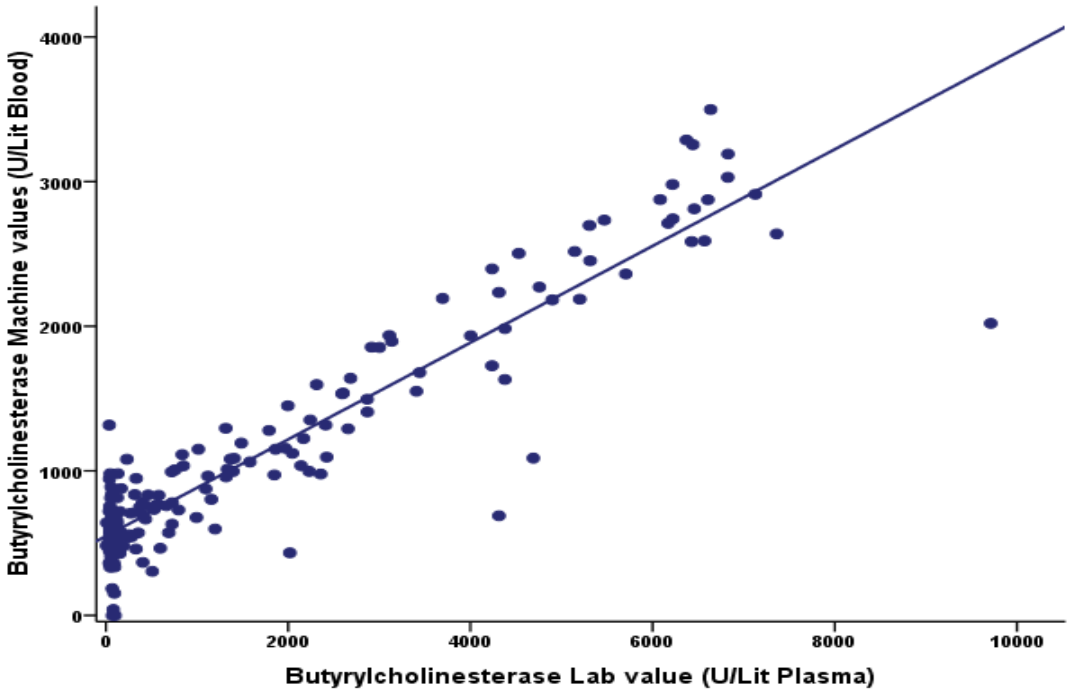


Figure 8-Correlation of BChE between two methods among patients with OP poisoning

2.3 CORRELATION OF AChE AMONG NORMAL CONTROLS (Figure 9)

Figure 9 shows the correlation among normal controls and the correlation coefficient is 0.259(p=0.093) which suggest a poor correlation between Lab AChE and Machine AChE among normal controls.

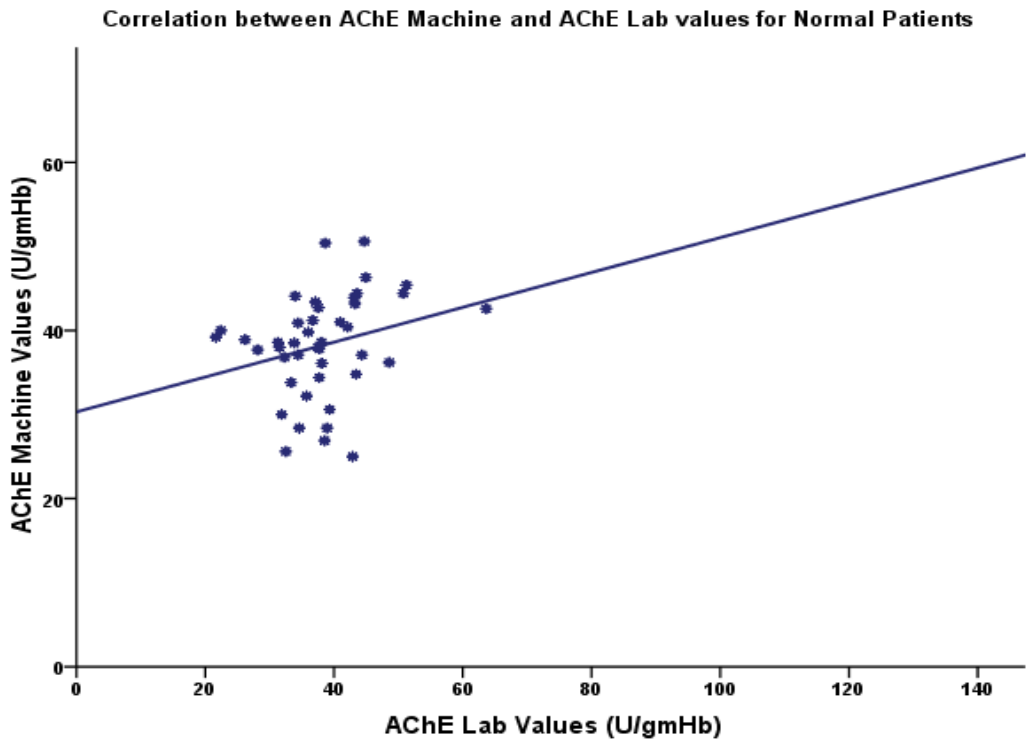


Figure 9-Correlation of AChE between lab and machine among normal controls

2.4 CORRELATION OF AChE AMONG NON OP PESTICIDE POISONING CONTROLS (Figure 10)

Figure 10 shows the correlation AChE between machine and lab among non OP controls

The correlation coefficient is -0.105 (p=.753) which suggested poor correlation of two methods among non OP controls.

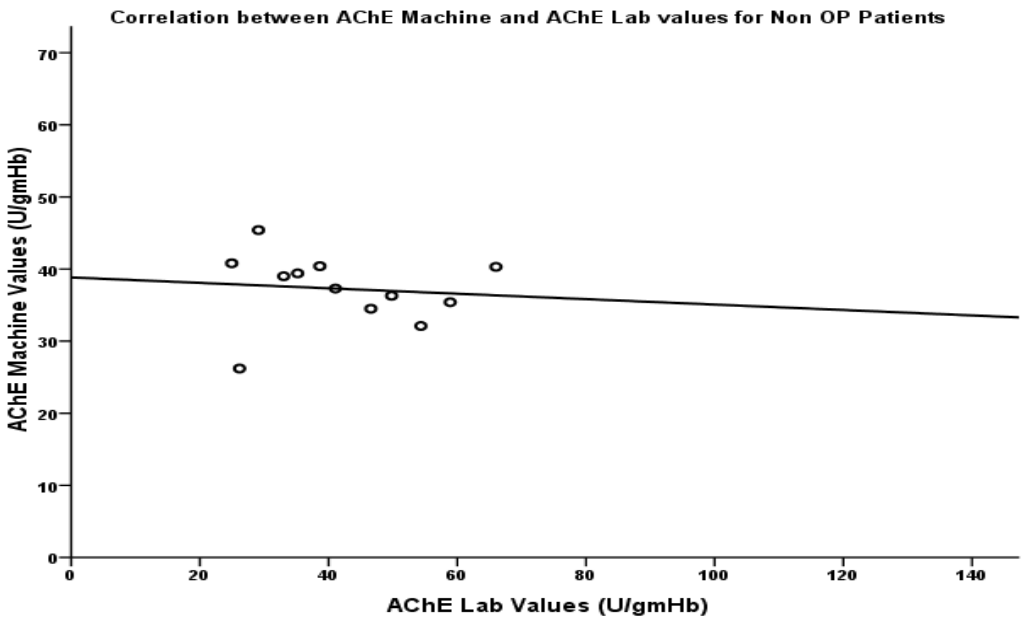


Figure 10-Correlation of AChE between lab and machine values among non OP pesticide controls

2.5 OVERALL CORRELATION OF BChE AMONG NORMAL CONTROLS AND NON OP PESTICIDE CONTROLS (Figure11 and 12)

Correlation of machine and lab values in detecting BChE levels among normal controls was not statistically significant (r2-0.300) (p=0.095).

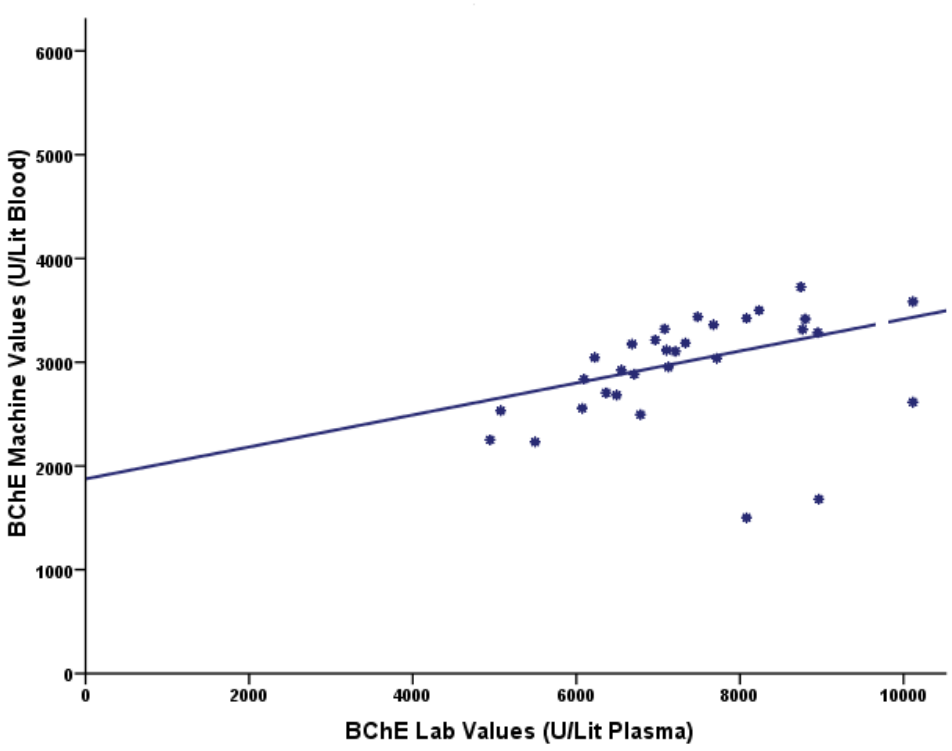


Figure 11-Correlation of BChE among normal healthy controls

Figure 12 shows **correlation of BChE between machine and lab among non OP poisoning controls**

The correlation coefficient was 0.158(p=0.709) which suggested poor correlation between BChE Machine values and BChE Lab values among Non OP pesticide poisoning.

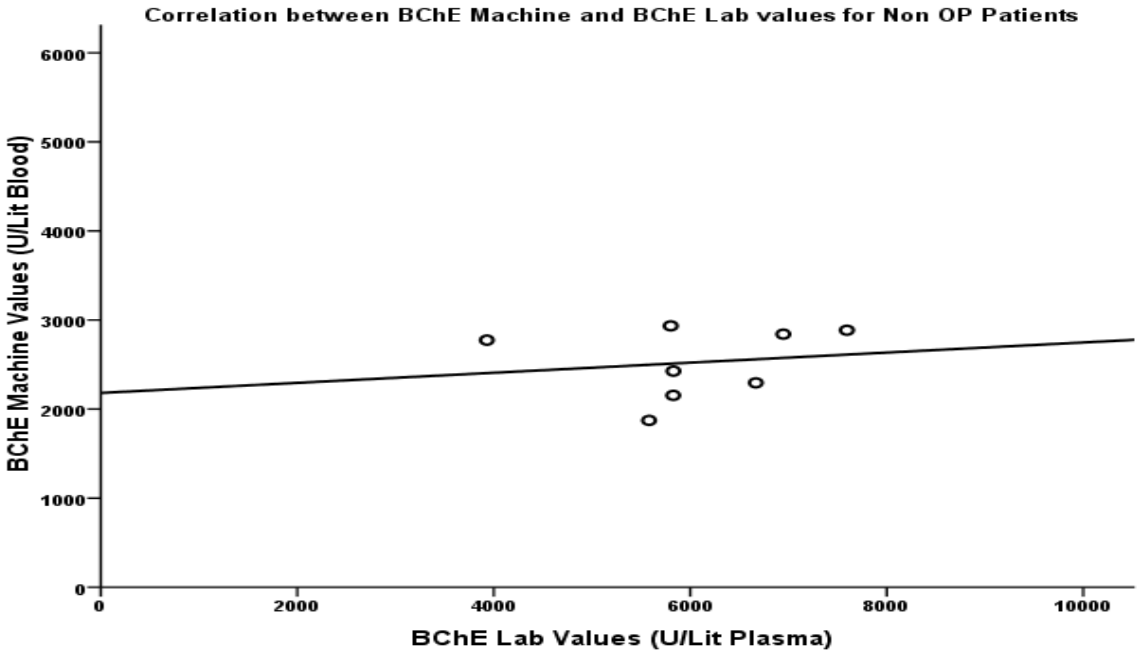


Figure 12-correlation of BChE between machine and lab among non OP poisoning controls

3.1 CORRELATION OF ACHE BETWEEN MACHINE AND LAB ON DIFFERENT DAYS OF HOSPITALISATION IN OP POISONING (Table 12)

There was good correlation between lab AChE and machine AChE at admission, 12 hours and daily for the first seven days among OP poisoning patients .

Table 12-Correlation on different days for AChE

Variable	Correlation coefficient (R2)	Significance – P value
Correlation of Machine Ache with Lab Ache at admission	0.841	0.000
Correlation of Machine Ache with Lab Ache at 12 hours	0.905	0.000
Correlation of Machine Ache with Lab Ache on day2	0.958	0.000
Correlation of Machine Ache with Lab Ache on day 3	0.925	0.000
Correlation of Machine Ache with Lab Ache on day 4	0.786	0.000
Correlation of Machine Ache with Lab Ache on day5	0.877	0.000
Correlation of Machine Ache with Lab Ache on day 6	0.875	0.000
Correlation of Machine Ache with Lab Ache on day 7	0.873	0.000

3.2CORRELATION OF BChE BETWEEN MACHINE AND LAB ON DIFFERENT DAYS OF HOSPITALISATION IN OP POISONING

Table 13 shows good correlation between lab BChE and machine BChE at admission, 12 hour and daily for the first seven days among OP poisoning patients.

Table 13-Correlation on different days for BChE

Variable	Correlation coefficient (R2)	Significance – P value
Correlation of Machine BChe with Lab BChE at admission	0.847	0.000
Correlation of Machine BChE with Lab Bche at 12 hours	0.954	0.000
Correlation of Machine BChE with Lab BChE on day2	0.975	0.000
Correlation of Machine BChE with Lab BChE on day 3	0.941	0.000
Correlation of Machine BChE with Lab BChE on day 4	0.933	0.000
Correlation of Machine BChE with Lab BChE on day 5	0.962	0.000
Correlation of Machine BChE with Lab BChE on day 6	0.971	0.000
Correlation of Machine BChE with Lab BChE on day 7	0.977	0.000

4.1 CORRELATION OF MACHINE AChE Vs LAB AChE CATEGORY IN DIFFERENT SEVERITY GROUPS OF OP POISONING AND ACCORDING TO TYPE OF COMPOUNDS (Table 14)

Overall correlation between two methods for measurement of AChE is not affected by severity of poisoning (Namba scale) or type of compounds (di-methyl, di-ethyl and S-alkyl).

Table 14-Correlation of AChE based on type of compounds and severity

Variable	Correlation of machine AChE Vs Lab Ache	P value
Severity	0.782	0.038
Mild	0.886	0.000
Moderate	0.799	0.000
Severe	0.869	0.000
Type of compounds	0.817	0.000
S-alkyl	0.961	0.000
Dimethyl	0.803	0.000
Diethyl	0.897	0.000

4.2CORRELATION OF MACHINE BChE Vs LAB BChE CATEGORY IN DIFFERENT SEVERITY GROUPS OF OP POISONING AND ACCORDING TO TYPE OF COMPOUNDS (Table 15)

Table 15 shows that the overall correlation between lab and machine BChE is not affected by the severity of poisoning (Namba scale) or type of compounds (di-methyl, di-ethyl and S-alkyl).

Table 15-Correlation of BChE based on severity and type of compounds

Variable	Correlation of machine BChE Vs Lab BChE	P value
Severity	0.771	0.025
Mild	0.941	0.000
Moderate	0.878	0.000
Severe	0.933	0.000
Type of compounds	0.839	0.000
S-alkyl	0.931	0.000
Dimethyl	0.943	0.000
Diethyl	0.910	0.000

The normal value of AChE and BChE for machine and laboratory was calculated based on mean and 2 Standard deviation of the normal healthy control values. The normal value of machine AChE ranged from 26.02U/gm Hb to 50.42 U/gm Hb (Mean±2SD (38.22 ± 12.2) and BChE ranged from 1593.82 U/litre of Blood to 4219.5 U/Litre blood (2906.66±1312.84). The Normal Value of laboratory AChE ranged from 22.83 U/gm Hb to 52.35 U/gm Hb (38.09± 15.26) and BChE ranged from 4791.39 U/Litre of plasma to 9962.11 U/litre of plasma (7376.75±2585.36).

PART 3-CLINICAL CORRELATION STUDY OF POINT OF CARE AChE ESTIMATION

CLINICAL COHORT STUDY

Fifty nine patients were recruited for the clinical cohort study. They were followed up for seven days or till discharge along with serial measurement of BChE and AChE levels using rapid check mobile.

TIME TREND ANALYSIS

Serial AChE and BChE level's relationship with different variables were assessed using trend analysis using GEE (Generalized Estimating Equation regression analyses) method .The important variables considered for the trend analysis are

1. Severity,
2. Development of intermediate syndrome,
3. Mechanical ventilation and
4. Death.

The absolute rise of Mean AChE and BChE values on different days from the baseline was compared among patients with organophosphorus poisoning however the rise was not significant.

OVERALL TREND OF MACHINE VS LAB ACHE AND BCHE OVER TIME FOR PATIENTS WITH ORGANOPHOSPHORUS POISONING

Figure 13 and 14 show means AChE and mean BChE of machine and laboratory values over time. The time trend for both AChE and BChE shows that the machine and laboratory values parallel each other. Trend also shows that the mean AChE and mean BChE do not increase over time in the first seven days. Mean AChE and mean BChE machine values were lower than the laboratory values.

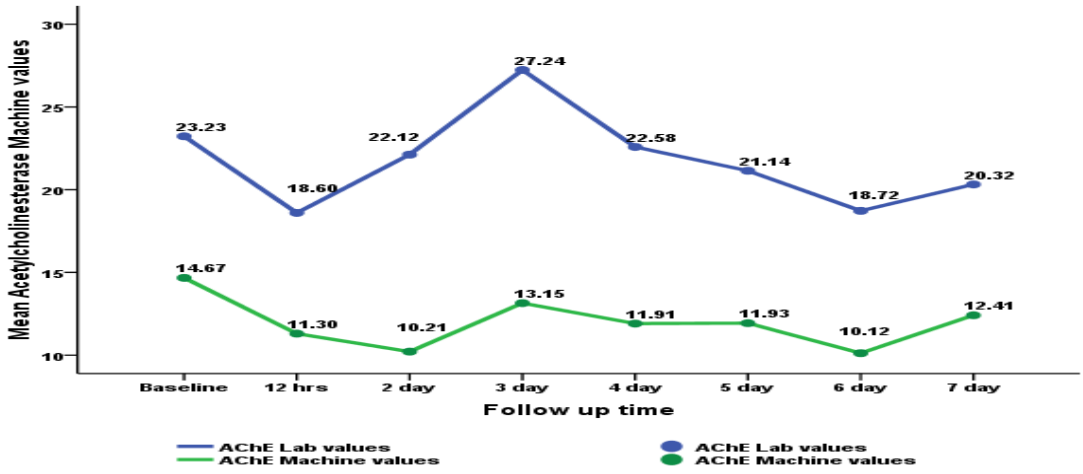


Figure 13-Mean AChE Machine values and AChE Lab values over a time

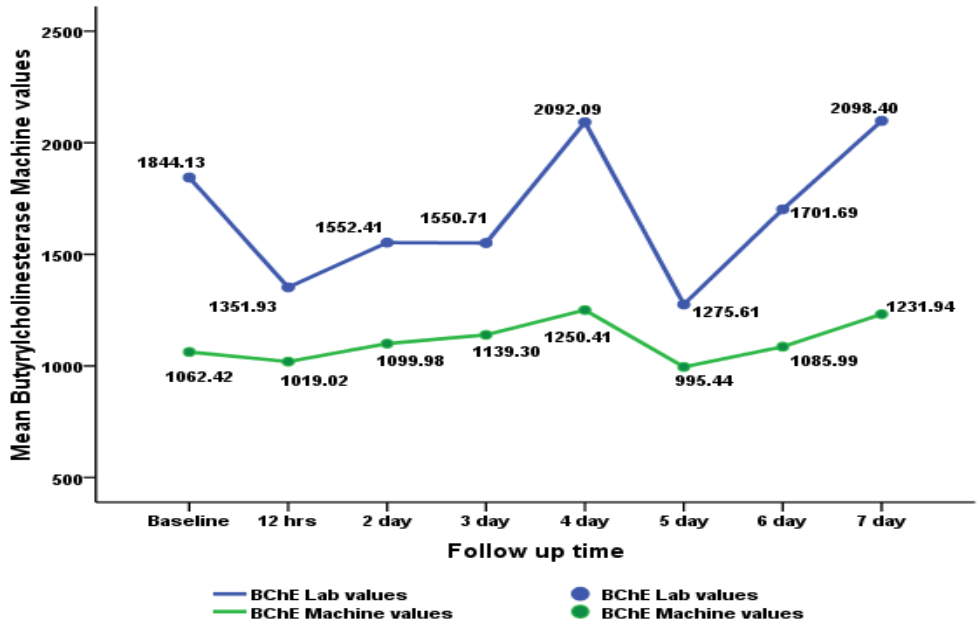


Figure 14-Mean BChE Machine values and BChE Lab values over a time

TEMPORAL PROFILE OF AChE AND BChE FOR SEVERITY

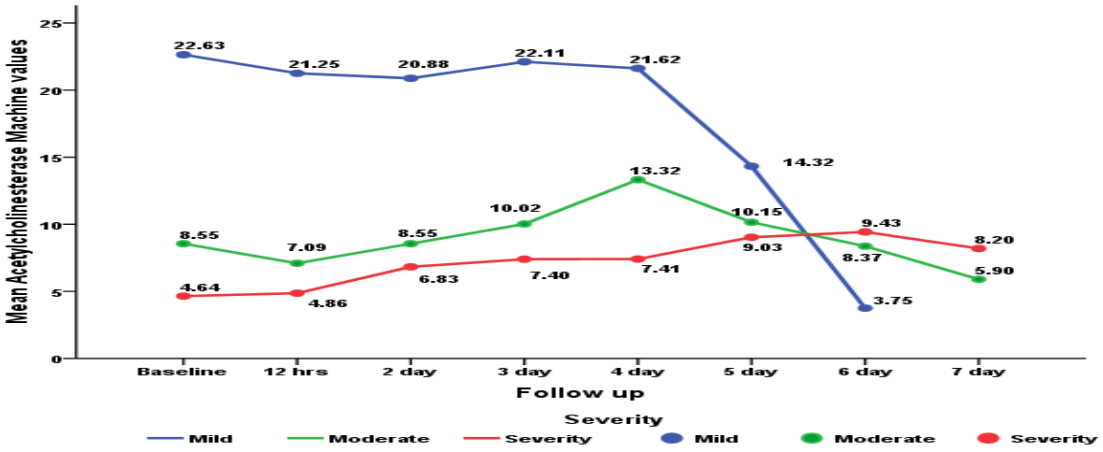


Figure 15-Temporal profile of AChE over time for severity

Figure 15 shows the graphical representation of trend analysis using GEE.

We have found that there was a significant inhibition of AChE levels among people with moderate and severe poisoning compared with people who had mild poisoning. Study shows that moderate poisoning group had AChE levels 11.67U/gm Hb less

than mild poisoning group over time with p value of 0.0004 and confidence interval of (-24.01 – (-4.47)). Similarly severe poisoning group had AChE levels 14.24U/gm Hb less than mild poisoning group over time with p value of p<0.02 and confidence interval of CI(-21.23 – (-2.12)).

The temporal profile of AChE in the mild poisoning group compared to temporal profile of moderate and severe poisoning groups showed mild inhibition of enzyme levels throughout the first 5 days. After 5 days, the change in pattern of graph among the mild poisoning group is probably due to the early discharge of these patients.

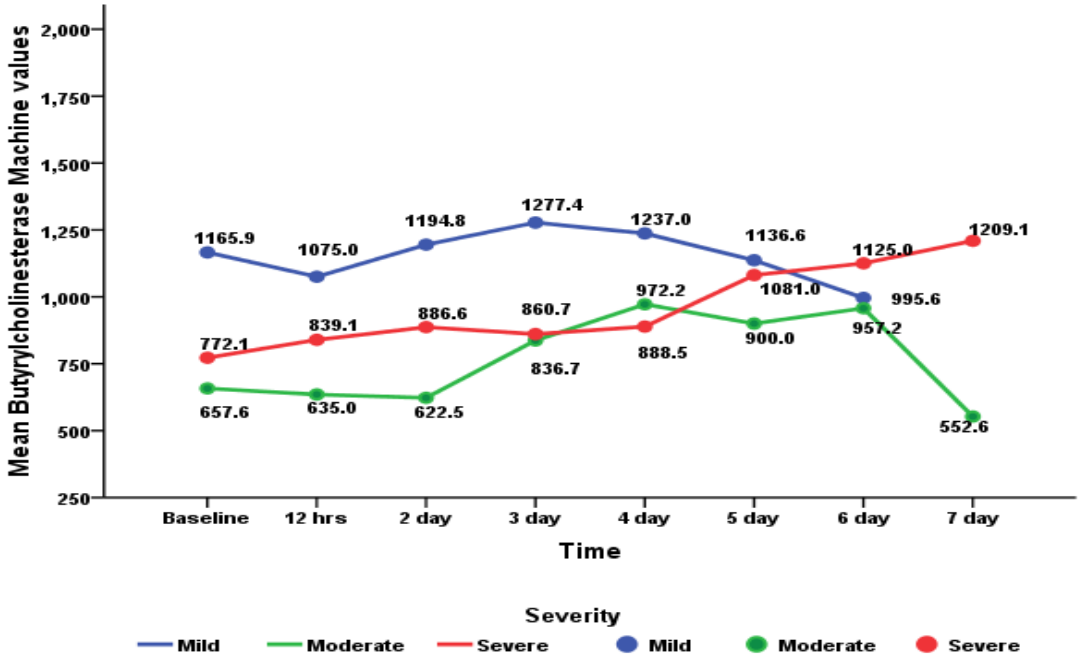


Figure 16-Temporal profile of BChE over time for severity

Compared with mild poisoning group, the moderate poisoning group had Mean BChE levels of 418.09 U/L of blood less than the mild group with p value of 0.049 and confidence interval of ((-826.15)-(-10.04).

Mean BChE values among mild and severe poisoning were comparable and there was no significant difference in two groups over time.

TEMPORAL PROFILE OF AChE AND INTERMEDIATE SYNDROME

There were 17 patients who developed intermediate syndrome and the temporal profile of mean AChE was compared to with that of the 32 patients who did not develop intermediate syndrome.

Figure 17 shows temporal profile of mean AChE over time among people who developed intermediate syndrome compared with people who did not develop intermediate syndrome. There was persistent inhibition of AChE over time among patients who developed intermediate syndrome compared with people who did not develop intermediate syndrome. On an average AChE levels among patients who developed intermediate syndrome were 9.43 (CI-16.01,-2.81) U/gmHb less than those who did develop intermediate syndrome and this reduction was statistically significant ($p<0.005$).

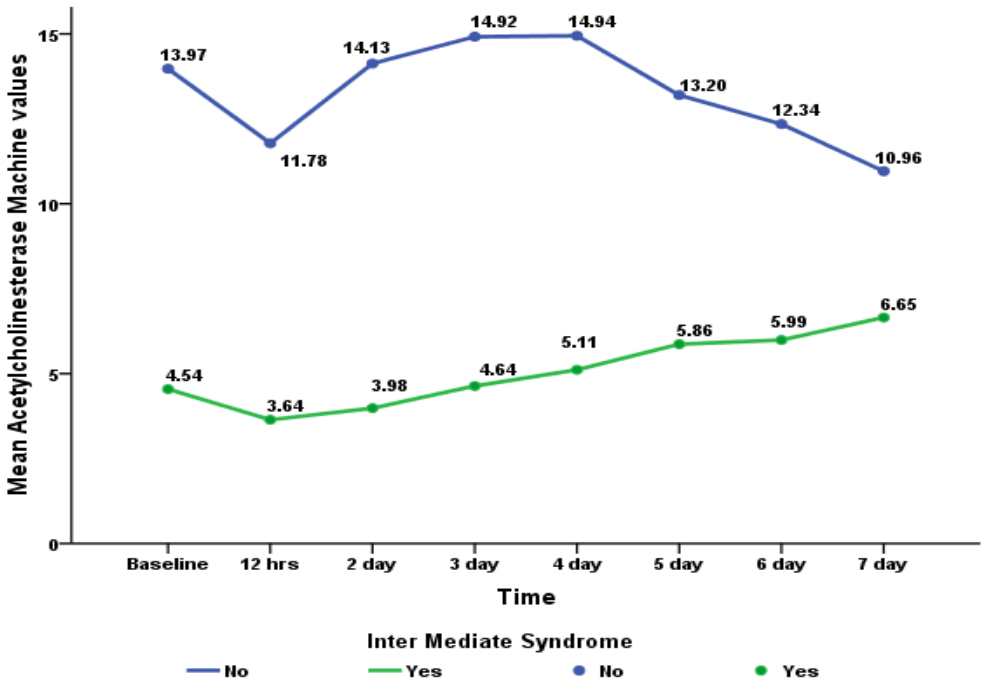


Figure 17-Temoral profile of AChE and intermediate syndrome

Figure 18-Shows temporal profile of BChE over time among people with intermediate syndrome. Mean BChE levels were low among patients with intermediate syndrome compared with those who did not develop intermediate syndrome. This difference was not statistically significant (p value 0.185)

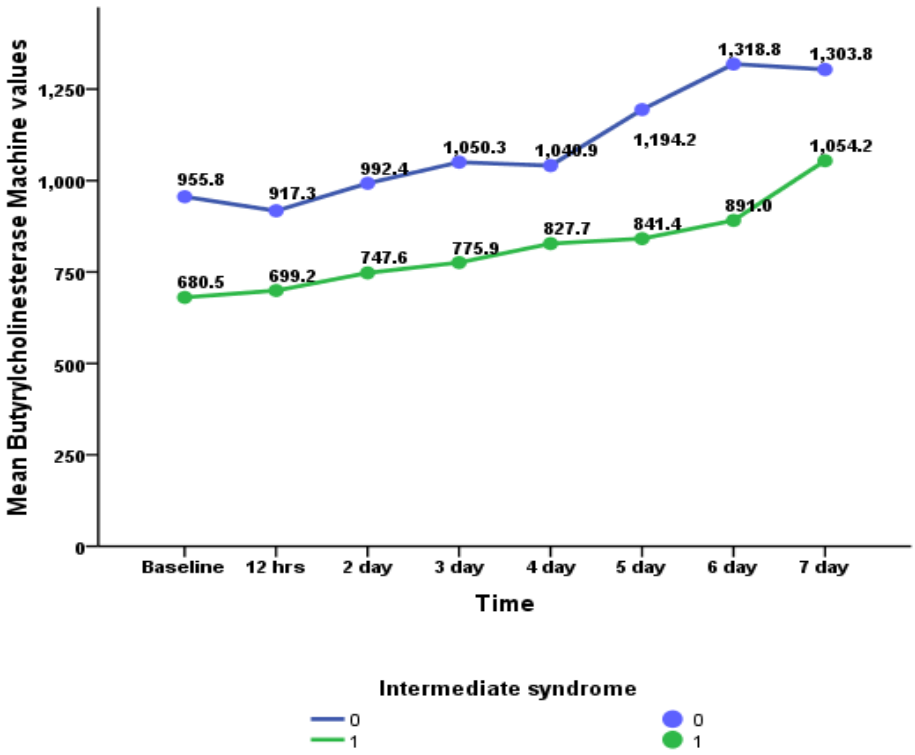


Figure 18-Temporal profile of BChE among patients for intermediate syndrome (0 denotes people who did not develop intermediate syndrome and 1- denote people who developed intermediate syndrome)

TEMPORAL PROFILE OF AChE AND BChE FOR VENTILATION

Figure -19 shows the mean difference of AChE between ventilated and non ventilated patients is 11.6 U/gm Hb across 7 days with 95 % confidence interval (-19.03 – (-4.11)) and p value of ($p<0.002$). Among ventilated patients, the mean AChE remained inhibited throughout the 7 days.

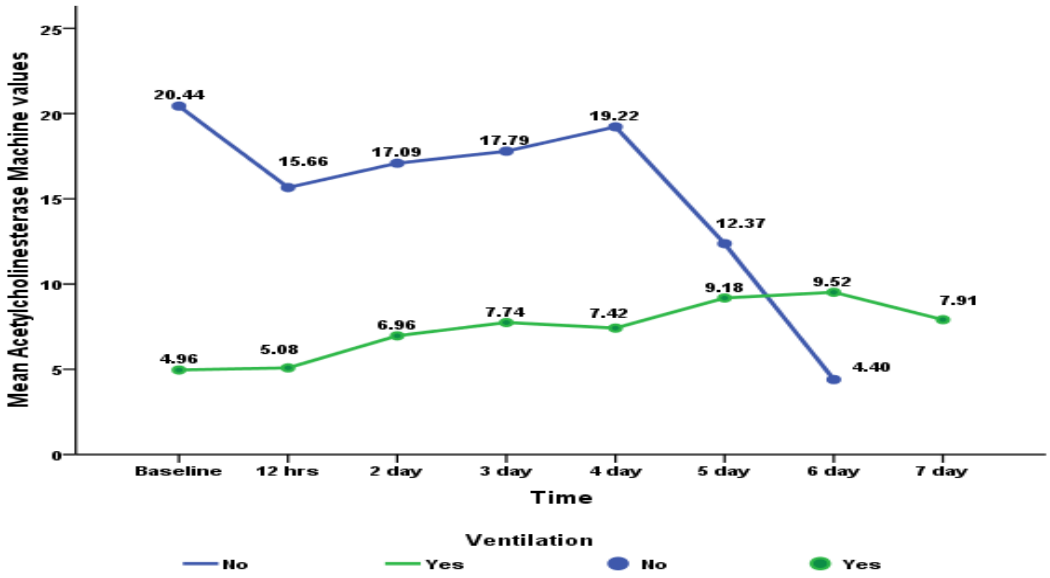


Figure 19-Temporal profile of AChE over time for Ventilation

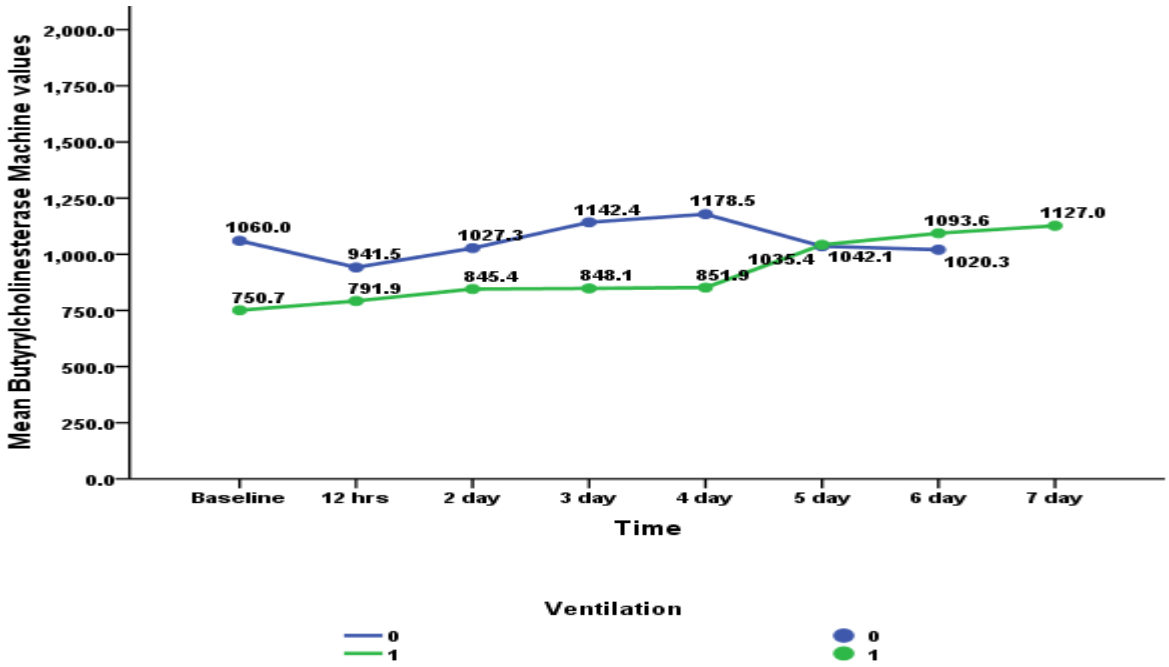


Figure 20-Temporal profile of BChE over time for Ventilation

A non statistically significant reduction (P value(0.245) in mean BChE enzyme levels was found on comparing people who required ventilation with those did not require ventilation over time.

TEMPORAL PROFILE OF AChE and BChE FOR DEATH

The figure 21 below shows that the mean AChE levels of the people who died remain persistently inhibited compared to those who survived. The difference in baseline mean AChE between patients who died and those who survived was 11.38 U/gm Hb which tended towards significance (p=0.000).

Similar significant reduction was not found in BChE levels (p value 0.059)

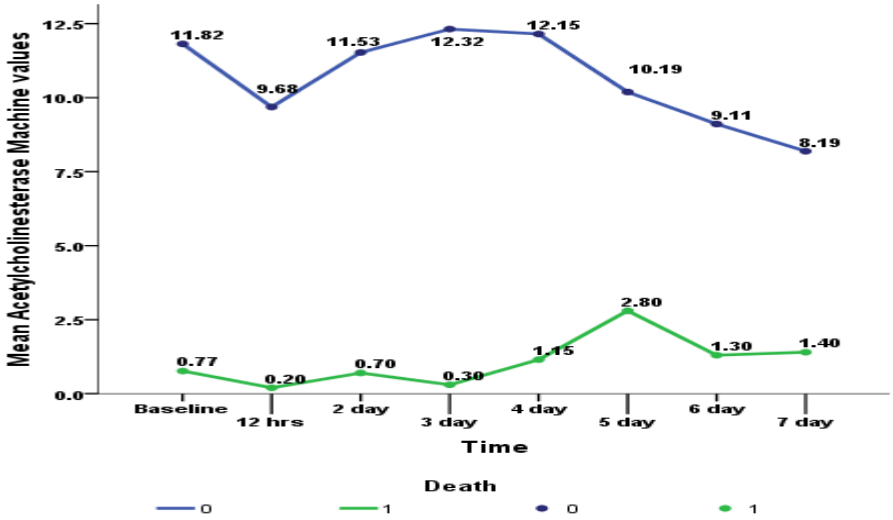


Figure 21-Temporal profile of AChE over time for death

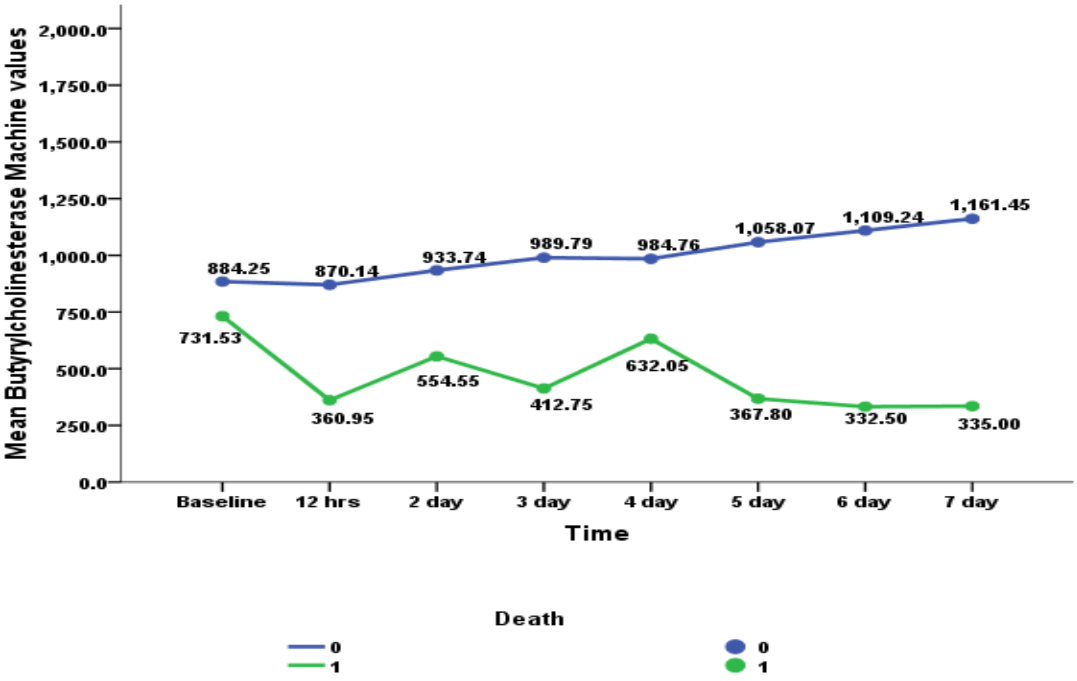


Figure 22-Temporal profile of BChE over time for Death

PREDICTION OF AChE AND BChE AND CLINICAL OUTCOME

The admission, day 3 and day 5 AChE and BChE values were correlated with:

1. Severity of poisoning
2. Development of Intermediate syndrome
3. Need for ICU admission
4. Need for ventilator support
5. Death
6. Duration of intermediate syndrome (less than 7 days and more than 7 days)
7. Duration of mechanical ventilation
8. Duration of hospitalisation (less than 7 days and more than 7 days
9. Type of compounds

CORRELATION OF ADMISSION MACHINE AChE, DAY3 AChE AND DAY 5 AChE AND CLINICAL OUTCOME. (Table 16)

Admission Machine AChE values showed good correlation with:

1. Severity of poisoning (p=<0.001)
2. Cholinergic crisis(p=.055)
3. Requirement of ICU admission (p=<0.001)
4. Requirement of mechanical ventilation (p=<0.001)
5. Development of intermediate syndrome (p=<0.021)
6. Death (p=0.067)
7. Hospital duration(p=0.001)

Day 3 Machine value of AchE also showed good correlation with severity of poisoning, need for ICU care, mechanical ventilation, development of intermediate syndrome, death and duration of hospitalisation. However day 3 AChE did not

correlate with the durations of intermediate syndrome, mechanical ventilation or ICU admission. Therefore Day 3 values could not predict the non-resolution of intermediate syndrome and the need for continued ventilation or ICU care.

Table 16-Correlation of clinical outcome with admission, day3 and day 5 AChE

Clinical variable	**AChE at Admission** Median (IQR)	**P value**	**AChE on Day 3** Median (IQR)	**P value**	**AChE on Day 5** Median (IQR)	**P value**
Ventilation duration						
Less than 7 days	1.6 (0.4 , 5.1)	0.620	2.1 (0.6 , 13.4)	0.750	4.40(2.57 , 14.7)	0.124
More than 7 days	1.6 (0.2 , 2.9)		1.7 (1.3 , 2.5)		2.80(1.95 , 3.7)	
Intermediate syndrome						
Less than 7 days	2.7 (0.9 , 7.4)	0.143	1.8 (1.3 , 2.7)	0.421	7.95(2.7 , 23.2)	0.055
More than 7 days	0.4 (0.0 , 3.8)		1.5 (0.6 , 2.1)		2.80(1.9 , 4.9)	
ICU duration						
Less than 7 days	1.7 (0.4 , 6.1)	0.570	2.1 (0.6 , 13.4)	0.723	5.05(2.6 , 14.7)	0.124
More than 7 days	1.6 (0.2 , 2.9)		1.7 (1.3 , 2.5)		2.80(1.9 , 3.6)	
ICU admission						
No	17.8 (4.2 , 30.5)	<0.001	9.4 (3.6 , 33.7)	0.001	6.10(3.6 , 23.2)	0.227
Yes	1.7 (0.3 , 4.3)		2.0 (1.0 , 9.6)		3.10(2.3 , 11.9)	
Cholinergic Crisis						
No	9.5 (2.6 , 39.2)	0.055	6.6 (2.5 , 37.2)	0.136	5.10(3.1 , 22.9)	0.341
Yes	2.4 (0.9 , 14.6)		2.9 (1.3 , 14.9)		3.25(2.3 , 12.1)	
Tracheostomy						
No	1.1 (0.3 , 1.9)	0.432	1.4 (1.2 , 1.8)	0.287	3.55(2.7 , 13.2)	0.039
Yes	1.8 (0.3 , 4.2)		2.1 (0.6 , 13.7)		2.60(1.0 , 2.8)	
Intermediate syndrome						
No	5.3 (1.3 , 21.3)	0.021	7.8 (2.3 , 23.6)	0.002	3.40(2.6 , 6.4)	0.052
Yes	0 .6 (0. 2 , 5.7)		11.7 (0.9 , 2.6)		2.20(0.8 , 2.8)	
Ventilation						
No	17.8 (5.1 , 30.5)	<0.001	10.7 (4.5 , 32.8)	<0.001	9.25(3.6 , 23.2)	0.171
Yes	1.6 (0.3 , 3.9)		1.9 (0.9 , 9.2)		3.10(2.4 , 10.6)	
Death						
No	3.9 (1.1 , 18.2)	0.067	4.5 (1.7 , 17.8)	0.019	4.00(2.4 , 12.4)	0.625
Yes	1.1 (0.0 , 1.2)		0.3 (0.0 , 0.6)		2.80(2.8 , 2.8)	
Hospital duration						
Less than 7 days	9.2 (1.6 , 21.4)	0.001	9.5 (3.2 , 28.8)	<0.001	11.40(4.0 , 25.0)	0.001
More than 7 days	1.5 (0.1 , 2.8)		1.7 (0.6 , 2.3)		2.80(1.5 , 4.8)	
Severity						
Mild	20.4 (5.7, 34.8)	<0.001	16.8 (5.7 , 40.9)	0.002	11.40(1.1 , 28.9)	0.175
Moderate	6.2 (1.5 , 16.1)		6.9 (2.7 , 17.6)		5.75(4.5 , 19.9)	
Severe	1.4 (0.2 , 2.6)		1.8 (0.8 , 6.8)		2.80(1.9 , 10.6)	
Type of compounds						
S , alkyl	4.0 (.00 , 18.7)	0.570	3.5 (0.7 , 17.9)	0.949	2.80(2.5 , 25.0)	0.354
Diethyl	3.6 (1.3 , 17.1)		4.1 (1.9 , 13.6)		5.10(2.5 , 12.1)	
Dimethyl	9.7 (0.9 , 22.9)		3.3 (1.3 , 26.6)		1.55(0.3 , 2.8)	

CORRELATION OF ADMISSION MACHINE BChE, DAY 3 BChE AND DAY 5 BChE AND CLINICAL OUTCOME. (Table 17)

Admission BChE did not have a role in predicting the clinical outcome and day 3 and day 5 BChE did not have any role in determining the duration of mechanical

ventilation ICU stay or intermediate syndrome. However patients with low BChE at admission, on day1 and day 3 had prolonged hospital stay. Day 3 and day 5 BChE levels correlated significantly to the type of compounds.

Table 17-Correlation of clinical outcome with admission, day 3 and day 5 BChE

Clinical variable	**BChE at Admission** Median (IQR)	**P value**	**BChE on Day3** Median (IQR)	**P value**	**BChE on Day5** Median (IQR)	**P value**
Ventilation duration						
Less than 7 days	628.2 (427.3837.2)	0.609	587.3 (308.5 , 1014.7)	0.330	854.8(586.6 ,1352.9)	0.599
More than 7 days	585.8 (473.4 , 915.4)		708.0 (505.8 , 1085.5)		822.8(525.9 , 1185.7)	
Intermediate syndrome						
Less than 7 days	755.5 (487.5 , 914.8)	0.191	617.6 (458.6 , 1081.8)	0.615	912.4(661.0 , 1366.0)	0.228
More than 7 days	498.2 (453.6 , 612.1)		577.1 (418.5 , 1006.2)		641.0(525.9 , 1033.8)	
ICU duration						
Less than 7 days	657.3 (439.5 , 791.3)	0.658	591.3 (388.2 , 1014.8)	0.468	854.8(633.9 , 1352.9)	0.430
More than 7 days	585.8 (473.5 , 915.4)		708.0 (505.8 , 1085.5)		822.8(525.9 , 1185.7)	
ICU admission						
No	717.3 (552.4 ,1293.5)	0.090	835.5 (650.0 , 1494.8)	0.137	751.0(583.2 , 1357.6)	0.974
Yes	657.3 (459.4 , 827.0)		617.6 (464.5 , 1081.8)		842.5(573.5 , 1300.1)	
Cholinergic Crisis						
No	691.0 (480.8 , 834.0)	0.742	785.3 (508.8 , 990.5)	0.730	609.1(301.8 ,768.8)	0.040
Yes	689.5 (486.2 , 953.9)		718.9 (562.9 , 1270.5)		871.1(633.9 , 1352.9)	
Tracheostomy						
No	673.5 (452.9 , 915.7)	0.364	591.3 (361.0 , 1081.8)	0.655	854.8(559.5 , 1328.6)	0.610
Yes	512.1 (459.3 , 691.0)		708.0 (552.9 , 944.3)		609.1(558.1 , 1087.6)	
Intermediate syndrome		0.141		0.135		0.095
No	703.6 (521.4 , 027.0)		812.9 (581.0 , 1429.8)		719.1(575.3 , 1316.5)	
Yes	543.9 (459.3 , 821.9)		605.3 (448.1 , 1013.1)		583.6(447.3 ,901.27)	
Ventilation						
No	713.3 (618.6 , 293.5)	0.064	841.3 (692.7 , 1462.3)	0.260	751.0(661.0 , 1357.6)	0.607
Yes	599.2 (459.3 , 867.9)		603.3 (453.4 , 1004.7)		842.5(564.9 , 1300.1)	
Death						
No	690.4 (483.7 , 915.7)	0.972	784.1 (579.6 , 1216.5)	0.081	832.6(600.6 , 1325.8)	0.131
Yes	571.5 (537.0 , 1086.1)		412.7 (361.0 , 464.5)		367.8(367.8 , 367.8)	
Hospital duration						
Less than 7 days	709.9 (585.4 ,1188.5)	0.028	824.2 (613.3 , 1523.5)	0.022	867.2(685.8 , 1524.4)	0.034
More than 7 days	540.5 (447.2 , 819.5)		596.3 (348.7 , 955.5)		675.3(515.5 , 1011.1)	
Severity						
Mild	811.6 (531.8 , 1631.6)	0.117	904.8 (789.2 , 1636.4)	0.032	705.0(522.0 , 1966.9)	0.976
Moderate	654.4 (439.1 , 746.1)		750.9 (562.2 , 1352.7)		751.0(633.9 , 1302.6)	
Severe	599.2 (470.9 , 847.5)		601.2 (461.6 , 963.4)		867.2(564.9 , 1300.1)	
Type of compounds						
S, alkyl	783.2 (501.9 , 1440.7)	0.077	691.6 (498.9 , 844.20)	0.001	1316.5(1022.6, 1613.9)	<0.001
Diethyl	651.7 (446.2 , 769.5)		611.5 (434.5 , 790.33)		655.4(535.9 , 809.3)	
Dimethyl	783.2 (564.6 , 1291.7)		1208.4 (917.4 ,1636.4)		462.9(367.8 , 558.1)	

Note: IQR – Inter quartile range, Mann-Whitney U was used

PART 4: DIAGNOSTIC ACCURACY OF AChE

Diagnostic Value of AChE in differentiating OP poisoning from Non OP poisoning

Diagnostic accuracy of admission AChE in differentiating OP poisoning from Non OP pesticide poisoning was assessed using 2 by 2 table analysis. For differentiating Organophosphorus poisoning patients from non organophosphorus poisoning Machine RBC AChE value of ≤ 25 U/gm Hb was taken as cut off. This value is the lower limit of 2SD for the normal population which we determined from our study.

From our study it was found that value of AChE levels of ≤ 25U/gm Hb has 84.75% sensitivity and 100% specificity in diagnosing a patient with OP poisoning with positive predictive value of 100% and Negative predictive Value of 57.14% and negative likelihood ratio of 0.15. Hence from this we can conclude that if the level of AChE less than 25 U/gm Hb, diagnosis of OP poisoning can be made with high sensitivity and positive predictive value. Hence this can be used as a screening test in emergency department in case of unknown poisoning. In the 9 patients with OP poisoning with AChE levels more than 25 U/gm Hb it was found that they belonged to latent or very mild poisoning group and did not develop complications or require ICU admission.

Table 18-2 by 2 table for AChE and diagnosis of OP poisoning

AChE U/gm Hb	Poisoning		Total
	OP	Non OP	
≤25	50	0	**50**
>25	9	12	**21**
Total	**59**	**12**	**71**

Diagnostic accuracy of Machine RBC AChE in determining development of intermediate syndrome, ICU admission and Ventilation

We have taken admission AChE level of 10 U/gm Hb as a cut off for assessing its role in determining the development of intermediate syndrome, requirement of ICU stay and ventilation. This value of 10 U/gm Hb represents the highest value of AChE among the patients who developed intermediate syndrome.

Diagnostic Accuracy of admission AChE for predicting development of intermediate syndrome

Among the patients with Acute OP poisoning a value of ≤ 10 U/gm Hb has a high sensitivity of 94.12% and specificity of 42.86% in predicting the development of intermediate syndrome with a negative predictive value of 94.74% and negative likelihood ratio of 0.14.

Table 19-2 by 2 table for AChE and intermediate syndrome

AChE	Intermediate syndrome		Total
	Yes	**No**	**Total**
≤ 10 U/gm Hb	16	24	**40**
>10 U/gm Hb	1	18	**19**
Total	**17**	**42**	**59**

Diagnostic accuracy of admission AChE for predicting requirement of ICU stay

Among the patients with Acute OP poisoning value of AChE levels of ≤10 U/gm Hb has a sensitivity of 85.71% and specificity of 58.33% in predicting requirement of ICU admission with a high positive predictive value of 75.5% and negative predictive value of 73.68% with a negative likelihood ratio of 0.24 and positive likelihood ratio of 2.06. These results indicate the need for ICU admission can be predicted for patients with OP poisoning with an admission AChE level of ≤ 10 U/gm Hb.

Table 20- 2 by 2 table for AChE and Need for ICU admission

AChE	ICU		Total
	Yes	**no**	**Total**
</=10 U/gm Hb	30	10	**40**
>10 U/gm Hb	5	14	**19**
Total	**35**	**24**	**59**

Diagnostic accuracy of admission AChE for predicting requirement of Mechanical Ventilation

Among the patients with Acute OP poisoning value of AChE levels of ≤10 U/gm Hb has a sensitivity of 91.43% and specificity of 66.67% in predicting the need for ventilation with a positive predictive value of 80% and negative predictive value of 84.21% and a negative likelihood ratio of 0.13 and positive likelihood ratio of 2.74. OP poisoning patients with a low AChE level of ≤10 U/gm Hb has a high chance for requirement of mechanical ventilation compared with those who have AChE levels of more than 10 U/gm Hb.

Table 21- 2 by 2 table for Ventilation

AChE	**Ventilation**		**Total**
	Yes	**No**	
<10 U/gm Hb	32	8	**40**
>10 U/gm Hb	3	16	**19**
Total	**35**	**24**	**59**

DISCUSSION

CLINICAL CHARACTERISTICS OF OP POISONING

In this study the patients were predominantly young, with more men and patients were uneducated doing unskilled labour and farming. This demographic profile is similar to other studies from CMC and reported by Selvaraj, Kumar and Joshi.(44–47)

In this study diethyl compounds predominated followed by dimethyl compounds. A significant proportion of OP pyrethroid combinations were also ingested which are known to be associated with higher toxicity. The majority of OP compounds were WHO Class I and Class II compounds which WHO recommends for banning. However these compounds are still widely available in India. A range of 11 OP compounds were ingested. The most common compounds ingested were Triazophos, Chlorpyrifos, Profenofos and Monocrotophos. Comparison of other studies shows that the predominant compounds vary over time and according to region(46,49,50). An earlier study in 2010 from CMC showed dimethyl compounds predominated and monocrotophos was the commonest OP compound ingested.(32, 45)

Patients presented early to a health centre and received pre-hospital care and were admitted to CMC within 6 hours. This is similar to other studies(50). The cholinergic crises was similar to the known literature(46,48,50). The majority of patients had moderate and severe poisoning. Hence the patient profile was severely poisoned. This is similar to the poisoning profile reported from CMC and other centres. (46, 50)

The majority of patients received treatment with gastric lavage and atropine. None of the patients received PAM as it has been our institutional policy not to use PAM because of the lack of evidence supporting benefit. The majority of patients required ICU admission and mechanical ventilation. The average duration of ventilation was 7 days. This is similar to other studies in India.(51,52)

28.8% patients developed intermediate syndrome, and average duration of intermediate syndrome was 8 days. This is similar to earlier reports from CMC of intermediate syndrome rate of 37.5% (32) and reported incidence of Intermediate syndrome from other centres ranging from 9 -38 %.(31,46,48,50,53) . Incidence of Delayed organophosphate induced encephalopathy (DOPE) in our study was 3.4% was lower than earlier reported rates from CMC of 8.6%(35). The mortality rate of 5.08% was comparable to other studies in India. (46, 50)

VALIDATION STUDY

The main objective of this study was validation of RBC AChE check mobile among patients with acute organophosphorus poisoning. In our study we have found a very good correlation in measurement of AChE using the check mobile and standard laboratory method.(R2-0.868 and ICC of 0.86). For the measurement of BChE also there was good agreement between two methods and the correlation coefficient was 0.927. This was the first study which validated this machine among patients with acute organophosphorus poisoning. The RBC AChE showed excellent correlation with different grades of severity of poisoning, with different types of compounds and on different days of poisoning.

The manufacturer of this machine validated this study using 10 samples of patients with dimethoate and chlorpyrifos poisoning and they have found a correlation of 0.93 for AChE and 0.98 for BChE on comparing the machine values with standard laboratory methods. In their correlation study samples were collected in Sri Lanka and used for validation. However the clinical profile of the patients was not correlated to the AChE and BChE(54). In our study of OP poisoning we have studied poisoning with different compounds (diethyl, dimethyl and s-alkyl), different severity groups and through the duration of poisoning.

In a Sri Lankan study Rajapakse et al has validated a similar machine Testmate ChE 400 among 14 patients with acute organophosphorus poisoning and they used 93 samples for validation of AChE and 91 samples for BChE. They have found correlation of 0.87 for AChE and 0.76 for BChE (41). Our study had a larger sample size and equal or better correlation coefficients AChE (0.87) for and BChE (0.9).

The normal control estimation (40 hospital staff) established normal machine value and laboratory values which were comparable to the German population(55). All the non-OP pesticide poisoning had normal range values. In both the normal health controls and non-OP controls the machine and laboratory values were almost similar. However the correlation between machine and laboratory values for BChE and AChE for normal health controls and non-OP controls was not statistically significant. The RBC AChE check mobile has probably been developed to be more sensitive and reliable in detecting the levels of inhibited level of enzymes in OP poisoning. This is the likely reason for Machine showing better correlation with OP poisoned patients than in normal controls and non –OP poisoning.

CLINICAL COHORT STUDY

In this study there was reliable correlation between admission RBC AChE level and severity of poisoning, need for ICU care, mechanical ventilation, development of intermediate syndrome and death. Severely inhibited enzyme indicates a severely poisoned patient. The AChE check mobile can be used to triage patients. Patients who are severely poisoned patients /and or have very low AChE levels should be admitted in intensively monitored setting as they have risk of hemodynamic or respiratory deterioration. The correlation of RBC AChE to severity of poisoning has been shown in other studies(56,57). The RBC AChE levels correlate with neuronal enzyme activity hence the admission AChE actually reflect the level of neuronal AChE inhibition. Thiermann et al showed correlation of RBC AChE levels and blockade of neurotransmission(58). The present study is the first report of using the AChE check mobile to assess severity of poisoning.

In this study BChE did not show similar correlation to clinical outcomes. BChE levels are sensitive markers of OP exposure. However the level of inhibition does not correlate to neuronal AChE inhibition. Other studies have also shown that BChE levels do not correlate to clinical outcomes.(3)

This study showed that the AChE and BChE remain persistently inhibited through the poisoning with little increase over time. There was correlation with the level of AChE inhibition over time with severity of poisoning, development of intermediate syndrome and death. While these correlations of the temporal profile are significant,

they are likely to represent the level of initial inhibition of AChE on day 1 which persists. The temporal profile is unlikely to have prognostic significance.

Serial values of AChE on day 3 and day 5 did not predict the need for continued ventilation and prolonged intermediate syndrome. Patients who were weaned from the ventilator still had persistently inhibited AChE. AChE recovery takes an average time of 82 days as new red cells have to be synthesised for RBC AChE to recover (38).However neuronal AChE recovery is likely to be much faster. Therefore this study did not show the usefulness of serial monitoring of AChE in predicting neurological recovery from OP poisoning. Other studies too have not shown the benefit of serial measurement of AChE in managing patients with OP poisoning.

DIAGNOSTIC ACCURACY OF ADMISSION AChE

Diagnostic value of AChE in Poisoning

The RBC Check mobile is highly accurate in diagnosing OP poisoning and differentiating it from non-OP poisoning. At an AChE of ≤ 25U/gm Hb the test had a sensitivity of 84.75% and specificity of 100%. A value of ≤25 U/gm Hb rules is diagnostic of OP poisoning. The test has a low false negativity rate in mild and latent OP poisoning. Nine patients with OP poisoning had values more than 25 U/gm Hb, and among them 3 had latent poisoning and remaining 6 had mild poisoning and none of them developed complications.

Prognostic value of admission AChE

In the study we assess the diagnostic accuracy of admission machine AChE in predicting the development of intermediate syndrome, need for ICU care and mechanical ventilation. AChE level ≤ 10 U/gm Hb predicted intermediate syndrome (sensitivity 94.12%. specificity 42.86% , positive predictive value of 75.5% and a negative predictive value of 94.74%), ICU admission (sensitivity 85.71%, specificity 58.33%, positive predictive value 75.5% and negative predictive value of 73.68%) and need for mechanical ventilation (sensitivity 91.43%, specificity 66.67% positive predictive value of 80% and negative predictive value 84.21%) . These results suggest that machine AChE ≤ 10 U/gm Hb can be used to triage patients in emergency to decide need for ICU or high dependency admission for careful clinical monitoring.

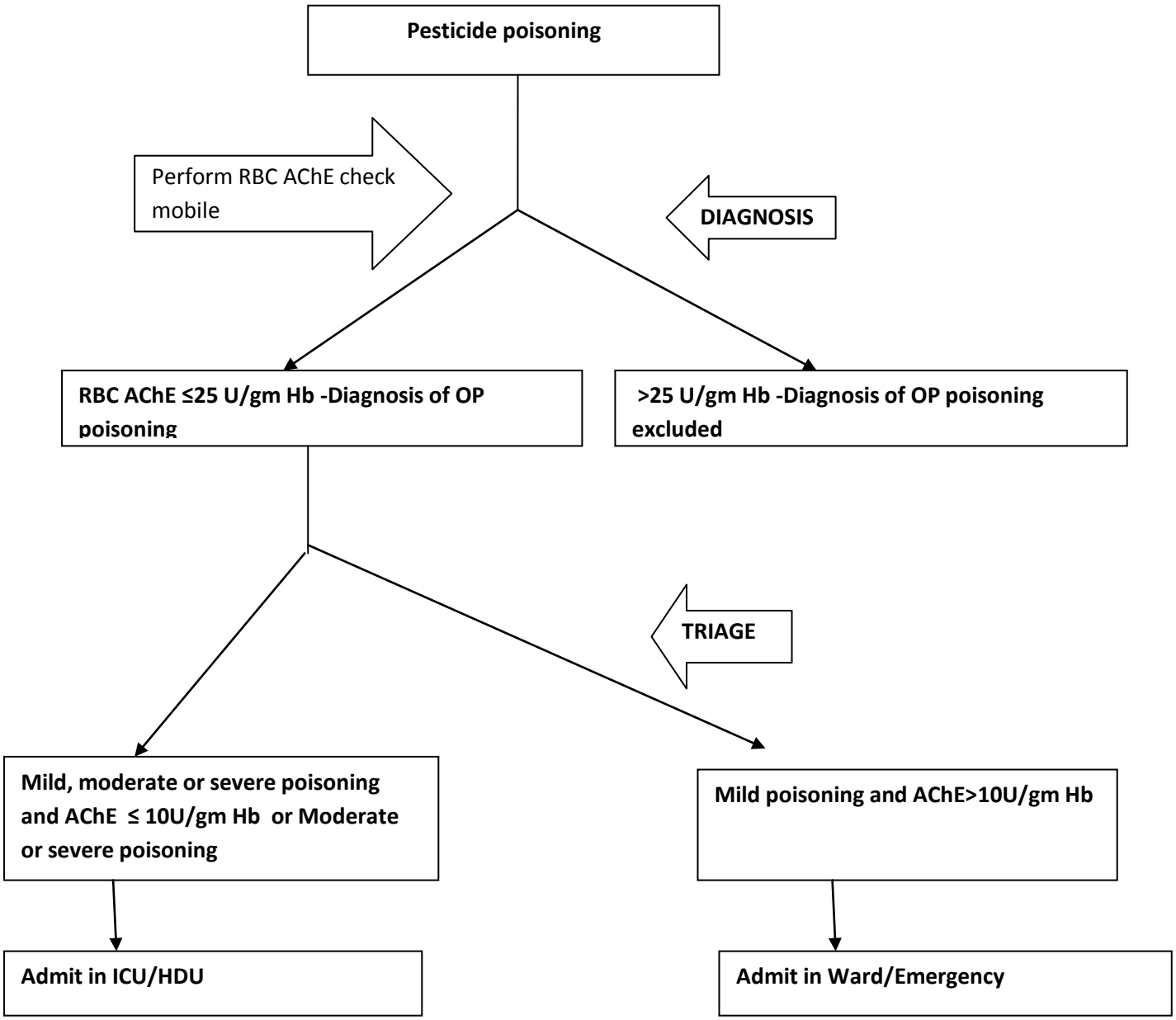
Hence from our data we can conclude that this machine can be used in two ways in the management of OP poisoning- a) Diagnostic test- To reliably diagnose OP poisoning and differentiate OP poisoning from non-OP pesticide poisoning.

b) Prognostic test regarding triage-Low values accurately predict development of complications and hence need for ICU care.

Both BChE and AChE estimations are not readily available in primary health centres, taluk and district hospitals which manage OP poisoning patients. This test can be done in the emergency at the bedside by clinical staff without significant technical expertise. The results are available in 5 minutes. There AChE check mobile can be used a point of care test in the emergency just like a glucometer or a pulse oximeter for diagnosis and prognostication.

Based on this we propose an algorithm for approach to diagnosis and management of OP poisoning using acetylcholine esterase Check mobile.

ALGORITHM FOR DIAGNOSIS AND TRIAGING OF OP POISOING USING THE RBC AChE CHECK MOBILE



IMPLICATIONS OF THIS STUDY

The study had demonstrated that the point of care AChE measurement can be reliably used to diagnose OP poisoning in India. This is a major advance as cholinesterase measurements both BChE and AChE are not widely available in the primary and secondary health centres where OP poisoning patients are taken care of. Even where tests are available the turn around time for results are slow and hence the results of the test cannot be used for clinical decision making. The RBC Check mobile can be useful in primary health centres, district hospitals and taluk hospitals (1) to diagnose OP poisoning and (2) for decision regarding triage Patients with very low AChE should be transferred to a set up with ICU care facilities equipped for mechanical ventilation.

FUTURE SCOPE AND RECOMMENDATIONS

1. To explore making this machine available in a cost-effective manner in the government health system.
2. Training of emergency department and medicine department doctors in the use of this machine.
3. A field level feasibility study at primary and secondary hospital level in diagnosing and managing OP poisoning.
4. Evaluating the proposed triage algorithm using AChE check mobile measurement.

CONCLUSIONS

- In this study RBC AChE check mobile a point of care testing has been validated against standard laboratory method among patients with acute OP poisoning. The RBC AChE check mobile shows excellent agreement with laboratory measurement both for detecting RBC AChE and BChE.
- This point of care testing can be reliably used to differentiate patients with OP poisoning from patients from non-OP pesticide poisoning with a sensitivity of 84.75% and sensitivity of 100%.
- Severely inhibited RBC AChE levels predict severe OP poisoning, need for ICU admission, mechanical ventilation and development of intermediate syndrome.
- RBC AChE measurement by RBC Check mobile point of care testing can help triage patients with Organophosphorus poisoning. Very low levels of RBC AChE (≤10U/gm Hb) predict the need for mechanical ventilation and intermediate syndrome. Hence such patients can be admitted to a carefully monitored set up or ICU. Patients with RBC AChE levels >10 who do not have clinically severe poisoning can be admitted into the ward.
- Serial AChE or BChE measurement is not useful to determine recovery from poisoning and predict duration of intermediate syndrome or need for continued mechanical ventilation.

LIMITATIONS OF THE STUDY

We have not included any patients with carbamate poisoning.

There are no other significant limitations to this study.

BIBLIOGRAPHY

1. WHO-The Impact of Pesticides on Health: Preventing Intentional and Unintentional Deaths from Pesticide Poisoning [Internet]. WHO; Available from: http://www.who.int/mental_health/prevention/suicide/en/PesticidesHealth2.pdf

2. Banerjee I, Tripathi S, Roy AS. Clinico-Epidemiological Characteristics of Patients Presenting with Organophosphorus Poisoning. North Am J Med Sci. 2012 Mar;4(3):147–50.

3. Eddleston M, Eyer P, Worek F, Sheriff MHR, Buckley NA. Predicting outcome using butyrylcholinesterase activity in organophosphorus pesticide self-poisoning. QJM Mon J Assoc Physicians. 2008 Jun;101(6):467–74.

4. Čolović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM, Vasić VM. Acetylcholinesterase Inhibitors: Pharmacology and Toxicology. Curr Neuropharmacol. 2013 May;11(3):315–35.

5. Radhika bobba, B.V.Venkataraman. Correlation between the severity of symptoms in organophosphorus poisoningand cholinesterase activity(Rbc and plasma) in humans. Indian J Pharmacol. 1996 40(3).

6. Thiermann H, Szinicz L, Eyer P, Zilker T, Worek F. Correlation between red blood cell acetylcholinesterase activity and neuromuscular transmission in organophosphate poisoning. Chem Biol Interact. 2005 Dec 15;157–158:345–7.

7. Devanur R.MM prasad. Relevance of Plasma Cholinesterase to Clinical Findings in Acute Organophosphorous Poisoning. ASIA Pac J Med Toxicol. 2(1):23–7.

8. Cholinesterase Kit for Field Diagnosis of Organophosphate Exposure [Internet]. medical corps international forum. Available from: http://www.mci-forum.com/cholinesterase-kit-for-field-diagnosis-of-organophosphate-exposure/

9. WHO | Suicide prevention-Preventing suicide a global initiative [Internet]. Available from: http://www.who.int/entity/mental_health/suicide-prevention

10. WHO | Quality of suicide mortality data [Internet]. WHO mental health; Available from: http://www.who.int/mental_health/suicide-prevention/mortality

11. Vikram Patel,. Suicide mortality in India: a nationally representative survey. Lancet. volume 379(june 23,2012).

12. National crime bureau. Suicides in India. ncrb.nic.in/statpublications/adsi/adsi2014;

13. Report on Medical certification of cause of death 2010. goverment of India;

14. WHO | Suicide data [Internet]. WHO. [cited 2014 Oct 16]. Available from: http://www.who.int/mental_health/prevention/suicide/suicideprevent/en/

15. IASP. Who Safer assess to pesticides- community interventions [Internet]. Who-Management of ental and brain disorders; 2006. Available from: www.who.int/mental_health/prevention/suicide/pesticides_safer_access.

16. Anisa Begum, Chrispal A, Ramya I, Reginald A, Zachariah A. Clinical profile of acute poisonings in the adult at Christia.

17. Marrazza G. Piezoelectric Biosensors for Organophosphate and Carbamate Pesticides: A Review. Biosensors. 2014 Sep 9;4(3):301–17.

18. Sanjay Upadhyay, Mukesh K. Sharma, Vepa K. Rao,, Bijoy K. Bhattacharya,. Organophosphorous Compounds- Toxicity and Detection Approach.

19. Eddleston M, Buckley NA, Eyer P, Dawson AH. Management of acute organophosphorus pesticide poisoning. Lancet. 2008 Feb 16;371(9612):597–607.

20. WHO recommended classification of pesticides by Hazard and guidelines to classification-2009. WHO-International programme for chemical safety;

21. Bateman DN, Dr RDJ, Dr JPT, Thomas SHL, Vale A. Oxford Desk Reference: Toxicology. Oxford University Press; 2014. 433 p.

22. Dvir H, Silman I, Harel M, Rosenberry TL, Sussman JL. Acetylcholinesterase: From 3D Structure to Function. Chem Biol Interact. 2010 Sep 6;187(1–3):10–22.

23. Fukuto TR. Mechanism of action of organophosphorus and carbamate insecticides. Environ Health Perspect. 1990 Jul;87:245–54.

24. Eddleston M, Szinicz L, Eyer P, Buckley N. Oximes in Acute Organophosphate Pesticide Poisoning: a Systematic Review of Clinical Trials. QJM Mon J Assoc Physicians. 2002 May;95(5):275–83.

25. Peter JV, Jerobin J, Nair A, Bennett A. Is there a relationship between the WHO hazard classification of organophosphate pesticide and outcomes in suicidal human poisoning with commercial organophosphate formulations? Regul Toxicol Pharmacol RTP. 2010 Jun;57(1):99–102.

26. Eddleston M, Eyer P, Worek F, Mohamed F, Senarathna L, von Meyer L, et al. Differences between organophosphorus insecticides in human self-poisoning: a prospective cohort study. Lancet Lond Engl. 2005 Oct 22;366(9495):1452–9.

27. Peter JV, Sudarsan TI, Moran JL. Clinical features of organophosphate poisoning: A review of different classification systems and approaches. Indian J Crit Care Med Peer-Rev Off Publ Indian Soc Crit Care Med. 2014 Nov;18(11):735–45.

28. Wadia RS, Sadagopan C, Amin RB, Sardesai HV. Neurological manifestations of organophosphorous insecticide poisoning. J Neurol Neurosurg Psychiatry. 1974 Jul 1;37(7):841–7.

29. Senanayake N, Karalliedde L. Neurotoxic Effects of Organohosphorus Insecticides. N Engl J Med. 1987 Mar 26;316(13):761–3.

30. Dr. Vikram Palimar,. Intermediate syndrome in organophosphorus poisoning. JIAFM 2005. 20005;27(1):0971–3.

31. A Zachariah, , L Tomas, M Alexander. Clinical electrophysiological and biochemical studies of intermediate syndrome in acute Organophosphorus poisoning.

32. M. Dandapani, A. Zachariah, M.R. Kavitha, L. Jeyaseelan & A. Oommen. Oxidative damage in intermediate syndrome of acute organophosphorous poisoning. 117(june 2003):253–9.

33. John M, Oommen A, Zachariah A. Muscle injury in organophosphorous poisoning and its role in the development of intermediate syndrome. Neurotoxicology. 2003 Jan;24(1):43–53.

34. Peter JV, Prabhakar AT, Pichamuthu K. Delayed-onset encephalopathy and coma in acute organophosphate poisoning in humans. Neurotoxicology. 2008 Mar;29(2):335–42.

35. Namba T, Nolte CT, Jackrel J, Grob D. Poisoning due to organophosphate insecticides. Am J Med. 1971 Apr 1;50(4):475–92.

36. Paul L.Knechtges. An Evaluation of Blood cholinesterase testing methods for military health surveilance. United States Army Center for Environmental Health Research Fort Detrick,; 2008.

37. Mason HJ. The recovery of plasma cholinesterase and erythrocyte acetylcholinesterase activity in workers after over-exposure to dichlorvos. Occup Med Oxf Engl. 2000 Jul;50(5):343–7.

38. EQM Research. Testmate ChE cholinesterase test system(Model 400).

39. Haigh JR, Lefkowitz LJ, Capacio BR, Doctor BP, Gordon RK. Advantages of the WRAIR whole blood cholinesterase assay: comparative analysis to the micro-Ellman, Test-mate ChE, and Michel (DeltapH) assays. Chem Biol Interact. 2008 Sep 25;175(1–3):417–20.

40. Rajapakse BN, Thiermann H, Eyer P, Worek F, Bowe SJ, Dawson AH, et al. Evaluation of the Test-mate ChE (cholinesterase) field kit in acute organophosphorus poisoning. Ann Emerg Med. 2011 Dec;58(6):559–564.e6.

41. Blattner K, Nixon G, Jaye C, Dovey S. Introducing point-of-care testing into a rural hospital setting: thematic analysis of interviews with providers. J Prim Health Care. 2010 Mar;2(1):54–60.

42. Blattner K, Nixon G, Dovey S, Jaye C, Wigglesworth J. Changes in clinical practice and patient disposition following the introduction of point-of-care testing in a rural hospital. Health Policy. 2010 Jun;96(1):7–12.

43. Rajapakse BN, Neeman T, Buckley NA. Effect of acetylcholinesterase (AChE) point-of-care testing in OP poisoning on knowledge, attitudes and practices of treating physicians in Sri Lanka. BMC Health Serv Res. 2014;14:104.

44. Peter JV, Jerobin J, Nair A, Bennett A, Samuel P, Chrispal A, et al. Clinical profile and outcome of patients hospitalized with dimethyl and diethyl organophosphate poisoning. Clin Toxicol Phila Pa. 2010 Nov;48(9):916–23.

45. T. Selvaraj, 1, , T. Sudharson. Demographic and Clinical Profile of Organophosphorus Poisoning cases in a Medical College Hospital, Tamil Nadu. Indian J Forensic Community Med. 2016 Jun;3(2):124–7.

46. Kumar SV, Venkateswarlu B, Sasikala M, Kumar GV. A study on poisoning cases in a tertiary care hospital. J Nat Sci Biol Med. 2010;1(1):35–9.

47. Subash chandra Joshi. Profile of organophosphorus poisoning at tertiary care hospital in Uttarakhand. J Indian Acad Forensic Med. 35,No 4(october-december).

48. Dayanand Raddi,. Clinical profile of organophosphorus poisoning in a tertiary care hospital. Indian J Basic Appl Med Researc H. 2014 Dec;4(1):14–22.

49. Sudhagar Mookkappan, 1. A clinico - epidemiological profile of organ ophosphorous compound poisoning , a retrospective study in a tertiary hospital, in Puducherry. IOSR. 2015 Sep;14(9):51–6.

50. Ahmed SM, Das B, Nadeem A, Samal RK. Survival pattern in patients with acute organophosphate poisoning on mechanical ventilation: A retrospective intensive care unit-based study in a tertiary care teaching hospital. Indian J Anaesth. 2014;58(1):11–7.

51. Banday TH, Tathineni B, Desai MS, Naik V. Predictors of Morbidity and Mortality in Organophosphorus Poisoning: A Case Study in Rural Hospital in Karnataka, India. North Am J Med Sci. 2015 Jun;7(6):259–65.

52. M M, D K, R N, S C, R M, Pandey S. The spectrum of intermediate synddrome following acute organophosphorus poisoning-Aretrospective cohort study froma teaching hospital in Chhattisgarh. J Evol Med Dent Sci. 2014 May 19;3(20):5561–6.

53. F.Woreck,M.Baumann,B.Pfeiffer,N.Aurbek,H.Thiermann. Choline esterase kit for field diagnosis of organophosphphate poisoning. CBRN medical defence international;

54. Securete. ChEcheck mobile Method and technical facts.

55. Bobba R, Venkataraman BV, Pais P, Joseph T. Correlation between the severity of symptoms in organophosphorus poisoning and cholinesterase activity (RBC and plasma) in humans. Indian J Physiol Pharmacol. 1996 Jul;40(3):249–52.

56. Brahmi N, Mokline A, Kouraichi N, Ghorbel H, Blel Y, Thabet H, et al. Prognostic value of human erythrocyte acetyl cholinesterase in acute organophosphate poisoning. Am J Emerg Med. 2006 Nov;24(7):822–7.

57. Thiermann H, Szinicz L, Eyer P, Zilker T, Worek F. Correlation between red blood cell acetylcholinesterase activity and neuromuscular transmission in organophosphate poisoning. Chem Biol Interact. 2005 Dec 15;157–158:345–7.

ANNEXURES

ANNEXEURE-1 PATIENT INFORMATION SHEET

EVALUATION OF RBC ACETYLCHOLINESTERASE POINT OF CARE TESTING USING ACHE RAPID CHECK MOBILE IN PATIENTS WITH ORGANOPHOSPHATE POISONING AND ITS CORRELATION WITH CLINICAL PROFILE

INFORMED CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

You are being requested to participate in a study involving Patients with Organophosphorus poisoning. Please read this information form carefully. Take time to ask as many questions as you want. The study personnel will explain any word or information you do not clearly understand.

DESCRIPTION OF THE STUDY

This study is regarding organophosphate poisoning which is the compound that you have consumed. This is a common poison in India and causes much difficulty in relation to need for hospital admission and high cost. An enzyme in the nerves and brain the name of which is acetylcholinesterase is inhibited by organophosphates and causes the different symptoms and complications of this poisoning.

Right now tests for diagnosis and monitoring of organophosphate poisoning by measuring the level of this enzyme are not freely available. A machine called Rapid Check mobile can monitor the activity of the Acetylcholinesterase at the bedside and within 5 minutes. This machine has so far not been used for monitoring patients with organophosphate poisoning.

In this study we are evaluating whether this machine can measure the enzyme as accurately as tests in the laboratory and also whether daily measurement can help in management of organophosphate poisoning.

The persons performing the blood tests will not be involved in your management. The management will be decided by your doctors although the results of the laboratory tests will be made available to them and could help in your management.

FORESEEABLE RISKS OR INCONVENIENCES

No risks or inconveniences are expected to you by participating in this study. Blood sample is usually collected at admission for BuChE measurement and this test will be done on the same sample. A small amount of the daily blood samples obtained for daily patient care and a small amount will be sent to the laboratory for daily BuChE and RBC AChE measurement. Some of the blood sent to the laboratory may be stored for further studies. The investigation will be done free of cost for you; you will not be required to pay any money at any stage for the study purpose.

REASONABLY EXPECTED BENEFITS

The result of your enzyme measurement will be made available to the doctors taking care of you this may benefit in timely management. You may discuss the same with your treating physician. You will not be given any financial or in kind rewards for participating in this study.

STUDY PARTICIPATION AND WITHDRAWAL

Your participation in this study is strictly voluntary. You have the right to leave this study at any time. Refusal to participate or study discontinuation will not result in any penalty, or compromise of your medical care, or loss of benefits.

DATA COLLECTED

If you consent to participate in this study, the study personnel will collect the following information about you: demographic data, a history of your illness, examination findings, results of your blood tests and details of your treatment while in the hospital.

Blood will be obtained by finger prick once every day and the measurement done with the rapid check machine.

CONFIDENTIALITY OF YOUR INFORMATION

Your personal data will remain strictly confidential. Only the study doctors will be able to match your data to your identity. If the results of this study are published, you will not be identified by name in any publication or presentation of results. The raw data will be made available to company which has manufactured the machine and provided it for this study at a reduced charge exclusively for research purposes.

For further clarifications if any contact Dr. ---------- , Phone number----------

ANNEXURE-2, INFORMED CONSENT FORM

EVALUATION OF RBC ACETYLCHOLINESTERASE POINT OF CARE TESTING USING ACHE RAPID CHECK MOBILE IN PATIENTS WITH ORGANOPHOSPHATE POISONING AND ITS CORRELATION WITH CLINICAL PROFILE

Hospital Number	Study ID	Participant's Name	Age

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ] (ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ] (iii) I understand that the Sponsor of the clinical trial, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ] (iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ] (v) I agree to take part in the above study. [ ] Name of the study participant / signatory:

Signature / Thumb impression: Investigator's signature:

Date: _____/_____/_____ Date: _____/_____/_____

Name of witness: _____

Witness signature:

Date: _____/_____/_____

For further clarifications if any contact Dr. ---------- , Phone number----------

---

ANNEXURE -3 PATIENT PROFORMA

Clinical research form Serial number

Case categorization:

1. Confirmed OP poisoning
2. Non-OP pesticide poisoning If non-OP pesticide poisoning Name poison:

Ascertainment criteria for OP poisoning (Tick appropriately):

1. Patients who present with history of pesticide poisoning with an identified OP compound.

2. Patients who present with history of pesticide poisoning with typical toxidrome of organophosphate poisoning, and low BuChE levels.

3. Patients who present without a history of pesticide poisoning but with typical toxidrome of OP poisoning and low BuChE levels.

Informed consent obtained: Yes/No

DEMOGRAPHIC DETAILS

1. Name:
2. Hospital ID:
3. Age:
4. Sex: 1.Male / 2.Female
5. Marital status: 1.married / 2. Single / 3. Others
6. Education: 1.Uneducated / 2.Educated

Profession: 1.Unskilled/2.Semi-skilled/3.Skilled COMPOUND

1. Compound identified: Yes / no
2. Compound name:
3. Method by which compound identified: Name given by patient/leaflet/bottle brought
4. Type of compound : 1. Dimethyl / 2. Diethyl / 3. Others
5. Percentage of compound :

6. Compound quantity(volume):

DETAILS REGARDING INGESTION

7. Time and date of consumption:

8. Time and date of First medical contact:

9. Time and date of arrival ay CMC:

10. Treatment given outside : 1. Skin decontamination / 2.Induced Emesis / 3. Gastric Lavage/

    4. Atropine / 5. PAM / 6.Intubation

11. Toxidrome: 1. Salivation / 2. Lacrimation / 3. Urination / 4. Defaecation / 5. Vomiting / 6.Seizures /

    7. Breathlessness / 8. Fasciculations / 9.Altered sensorium

12. Severity of poisoning by Namba scale: Mild/Moderate/Severe

CLINICAL FEATURE – ON ADMISSION IN E&D

1. GCS at presentatation:

2. Pupils size: PINPOINT / DILATED/ NORMAL (2-5MM)

3. Pulse rate at presentation:

4. Blood pressure at presentation:

5. Respiratory rate at presentation:

6. Saturation at presentation:

7. GRBS

8.Symptoms at presentation

Vomiting/diarrhoea/abdominal pain/salivation/sweating/ urinary incontinence

/drowsiness/breathing difficulty/seizures/bleeding/giddiness/altered sensorium/agitation/blurring of vision/ sedation/asymptomatic

9.Signs at presentation

Diaphoresis,Pupil size, Lung crepitations,Salivation,Frothing at mouth,Fasiculations/muscle weakness,Single breath count,Paradoxical breathing,Abdominal tenderness,Fever

Severity of poisoning (by Namba Scale): Mild/Moderate/Severe

10.Investigations

Laboratory BChE:

12.Serial clinical assessment

Heart rate	DAY1	2	3	4	5	6	7
Blood pressure							
GCS							
MUSCLE POWER- Shoulder Adduction							
Abduction							
Extension							
Flexion							
Elbow Flexion							
Extension							
Wrist Flexion							
Extension							

Small muscles of the hand							
MUSCLE POWER –Hip-extension Flexion Adduction							
Abduction							
Knee Flexion							
Extension							
Ankle Dorsiflexion							
Plantarflexion							
Neck Muscle weakness-neck holding time							
Respiratory muscle weakness							
Tidal volume							
Pressure support PEEP							

Single breath count							
Miosis							
Crepitations							
Salaivation							
Diarrhea							
Cholinergic crises present/absent							
Atropine infusion rate							
Total atropine dose							
Sedation present/absent							

Intermediate syndrome: Present/absent

Duration of intermediate syndrome:

13. Did the patient Required ICU admission

14.Date of admission date of discharge

15.Did the patient require ventilator support?

16.Date intubated Time intubated

17.Indication for mechanical ventilation

18.Duration of mechanical ventilation

19.Did the patient require tracheostomy yes no

20.Duration of ICU stay.

21.Complications

Cardiac arrest-yes/no

Respiratory arrest-yes/ no

Infective complications-yes/ no What infection:

Criteria for diagnosis of infection:

Chest x-ray

Cultures:

22. Treatment:

Gastric lavage at CMC: Yes/No Charcoal at CMC: Yes/No

Atropine: Yes/No Duration (in days): Total dose of atropine:

23.Death – yes/ no

24. Cause of death:Cholinergic crises,Ventilatory and airway problems:,Infections:,Others:

Summary data:

OP compound: ----------/ Unknown Concentration: Volume:

Severity of poisoning: Mild/Moderate/Severe,Cholinergic crises present/absent Duration of cholinergic crises:

Atropine duration: Total atropine dose: GCS at admission:

Duration of low GCS: Requirement of mechanical ventilation: Yes/No

Duration of mechanical ventilation: Intermediate syndrome: Yes/No

Duration of intermediate syndrome: Duration of hospitalisation

Final outcome: Alive/ Dead/ Discharged against medical advise

Laboratory measurements:

11.Serial monitoring of cholinesterase

Rapid Check Machine

	Day1			Day2	Day3	Day4	Day 5	Day 6	Day 7	At discharge
	0	12	24							
BuChE(IU/L)										
RBC AChE(U/gmHB)										

Laboratory measurement (for validation study only)

	Day1	Day2	Day3	Day4	Day5	Day6	Day6
BuChE							
RBC AChE							

Name of person who filled the form and signature:

ANNEXURE 4

STANDARD OPERATING PROTOCOL FOR DETERMINATION OF CHOLINESTERASE STATUS IN WHOLE BLOOD AND PLASMA

MATERIALS REQUIRED FOR THE ASSAYS

1) Phosphate buffer (= PP, 0.1 M, pH 7.4)

Solution 1: Na2HPO4*2H20, MW 177.9 (17.8 g in 1000 ml distilled water)

Solution 2: KH2PO4, MW: 136.1 (13.6 g in 1000 ml distilled water)

800 ml of solution 1 was mixed and adjusted with solution 2 to pH 7.4 with pH-meter and filtrate and stored in amber bottle at +4°C.

2) Diluting reagent for blood dilution: Distilled water

3) 5,5´-dithio-bis(2-nitrobenzoic acid) (= DTNB, Ellman´s reagent)

396.3 mg DTNB (MW 396.3, Sigma) was dissolved in 100 ml of phosphate buffer (0.1 M, pH 7.4) and stored aliquots at ≤-20°C. The concentration is 10 mM in the solution and 0.3 mM in the cuvet.

4) Acetylthiocholine iodide (=ASCh)

41.12 mg ASCh (MW 289.2, Sigma) was dissolved in 5.0 ml distilled water and stored aliquots at ≤-20°C. The concentration is 28.4 mM in the solution and 0.45 mM in the cuvet.

5) S-Butyrylthiocholine iodide (= BuSCh)

200.47 mg BuSCh (MG: 317.2, Sigma) was dissolved in 10.0 ml distilled water and stored in aliquots at ≤-20°C. The concentration is 63.2 mM in the solution and 1.0 mM in the cuvet.

6) Ethopropazine (selective BChE inhibitor) 20.94 mg ethopropazine hydrochloride (MW 348.94, Aldrich) was dissolved in 10 ml of 0.01 M HCl under stirring and mild heating and stored in aliquots at ≤-20°C. The concentration is 6.0 mM in the solution and 0.02 mM in the cuvet.

7) Transformation solution

200 mg K3[Fe(CN)6] (MW 329.2), 50 mg KCN (MW 65,1), and 1000 mg NaHCO3 (MW 84.0) were mixed with 1000 ml distilled water, followed by 0.5 ml Triton X-100 and stored in a tightly sealed amber glass bottle at ambient temperature.

8) Diluting reagent for test erythrocytes

100 µl Triton X-100 (Sigma) was added to 100 ml phosphate buffer (0.1 M, pH 7.4) and mixed thoroughly with final concentration of Triton X-100 at 0.1%

9) Test erythrocytes and test plasma

Blood was taken in EDTA tube, centrifuged (10 min, 3000 rpm) and transfer plasma into tube (—test plasma‖). The dilute packed erythrocytes with PP were mixed, centrifuged and supernatant was removed. This was repeated three more times. Washed, packed erythrocytes were diluted with 10 volumes diluting reagent for test erythrocytes and mixed. Aliquots of test erythrocytes and test plasma were transferred in separate tubes and stored at ≤-20°C.

10) Cuvets: Polystyrene cuvets (10 x 10 x 45 mm, e.g. VWR 634-0675)

MEASUREMENTS IN THE ASSAYS

a) Haemoglobin content

Sample preparation: 0.3 ml of the blood dilution and 2.7 ml of the transformation solution were mixed in a cuvet and extinction was read after 10 min against a reference cuvet containing only transformation solution.

Conditions: Wavelength: 546 nm, Temperature: Ambient, Extinction coefficient: 10.8 * 103M-1cm-1, Cuvets with 1 cm light path

Calculation: µM Hb* = - extinction (mA)

10.8* molarity refers to iron

b) Erythrocyte AChE activity

Conditions: Wavelength: 436 nm, Extinction coefficient: 11.28 * 103M-1cm-1, Temperature: 37°C, Cuvet volume: 3.16 ml, Recording time: 2 min, Cuvets with 1 cm light path

Substrate blank%:

- 3000 µl phosphate buffer (PP), 100 µl DTNB, 10 µl ethopropazine, 50 µl ASCh

- PP, DTNB and ethopropazine were added to cuvet, equilibrated for 10 min, ASCh was added and mixed

Sample:

- 2800 µl PP, 100 µl DTNB, 200 µl blood dilution (sample), 10 µl ethopropazine, 50 µl ASCh

- PP, DTNB, ethopropazine and sample were added to cuvet, equilibrated for 10 min, ASCh was added and mixed

Calculation:

$$\mu M/min = \frac{mA/min\ (sample) - mE/min\ (blank)}{11.28}$$

Referred to haemoglobin content:

$$\mu M/min/\mu mol\ Hb = \frac{AChE\ activity\ (\mu M/min) * 2\$}{\mu M\ Hb}$$

$ Factor for correction of different dilution in the determination of haemoglobin and AChE since the whole blood specimen has been diluted with 0.1ml of sample to 2.0ml with diluting reagent

c) Plasma-BChE activity:

Sample preparation:

Thawed plasma was centrifuged with high speed for 1-2 min to remove cryoprecipitate.

Conditions: Wavelength: 436 nm, Extinction coefficient: 11.28 * 103M-1cm-1, Temperature: 37°C, Cuvet volume: 3.16 ml, Recording time: 2 min, Cuvets with 1 cm light path

Substrate blank%:

- 3000 µl phosphate buffer (PP), 100 µl DTNB, 50 µl BuSCh

- PP, DTNB and ethopropazine were added to cuvet, equilibrated for 10 min(37°C) and reaction with BuSCh was started

Sample:

- 3000 µl PP, 100 µl DTNB, 10 µl sample(Plasma), 50 µl BuSCh

- PP, DTNB, ethopropazine and sample were added to cuvet, equilibrated for 10 min(37°C) reaction with BuSCh was started .

Calculation:

$$\mu M/min = \frac{mE/min\ (sample) - mE/min\ (blank)}{11.28}$$

Referred to plasma content:

mU/ml plasma= µM/min * dilution factor (=316)

Calculations and Explanation for Erythrocyte AChE status at CMC

Erythrocyte AChE Activity

we are using the Cobas calibrator for automated systems (cfas) with a stated cholinesterase activity using acetylthiocholine substrate.Sample or calibrator volume is 2 µl in a final volume of 272µl.Since we are using a calibrator not the molar extinction coefficient no correction is necessary for the reagent volumes. Results are in U/L that is µmol (S to P) /min/L of sample. (S to P) is substrate to product in AChE activity reaction

Haemoglobin estimation at CMC. The whole blood is diluted 0.3ml to 3.0 ml with transformation solution, this is a 1 in 10 dilution. Extinction coefficient is 10.8×10^3 $mol^{-1}cnm^{-1}$ a 1 mol/l solution of pure HB will have an absorbance of 10.8×10^3 mol/LHb in the sample is Absorbance sample / 10.8×10^3µmol/LHb is Absorbance sample / 10.8×10^{-3}µmol/LHb is Ab sample x 1000/10.8.This has to be multiplied by 10 for the actual value in the undiluted whole blood.

Final calculation (AChE activity/per unit of Hb)

Erythrocyte AChE in µmol/min/L divided by Haemoglobbin in µmol/L Hb of sample

Regression equation is The literature states that 1 g of Haemoglobin is equiv to 62.1 µmolHb hence to convert multiply lab values x 62.1

ANNEXURE 5 – STANDARD OPERATING PROTOCOL FOR RBC AChE CHECK MOBILE

Components of AChE check mobile

Components of the ChE check mobile assay kit for the on-site determination of AChE and BChE activity in whole blood.

First step – Entry of Patient details

- Place RBC check mobile horizontally
- Avoid direct sun light
- Switch-on
- Insert user name

- Insert patient data

Second step- Background value of cuvete measurement

1. Place white capped covets into covet slot

2. Press „NULL“(„ZERO“)

3. Remove covet, remove white cap and place covet into parking position

Third step- Measurement of haemoglobin

4. Take blood with 10µl EDTA capillary

5. Insert capillary into open cuvet

6. Screw cuvet with white cap and mix carefully until all blood is transferred from the Capillary into the cuvet

7.Place capped cuvet with blood sample into the cuvet slot Cave: Capillary must not be in the Optical path

8. Press „Hb“

After measuring the back ground value an icon for hemoglobin will be appeared in the screen.Venous blood is collected at this point using sterile precautions . Soon after the collection of blood in the syringe 10 microlitres of blood will be collected in 2 EDTA coated capillaries as well.

One capillary blood will be used for BuChE and one for AChE measurements.

Cuvete is now removed from the port, white cap is removed and the capillary for AChE Measurement will be added to the cuvete. Following which it is sealed with white cap and the

blood in the capillaries will be mixed with buffer reagents by shaking movements. All capillary blood has to be mixed with buffer and it's made sure by visualizing the clear capillary. The position of the capillary should be perpendicular to the surface so that it doesn't interfere with the Photometric measurements. .

Fourth step- Mixing the reagent with the blood

9.Remove cuvet, replace white cap by red reagent cap

10.Press „START" and rotate cuvet overhead simultaneously

After well mixing cuvete is replaced in the measuring port and the haemoglobin icon is pressed. This will show start icon again along with instruction for the next step in English.

Cuvee is removed from the port and a reagent cap which is red coloured for RBC AChE and yellow coloured for BuChE is agents. After exchanging the red coloured RBC AChE press the start button along with simultaneous mixing of blood with reagent through shaking movements. This will ensure that the blood is mixed with the reagent for 10 seconds

Fifth step- Enzyme measurement

11.Rotate cuvet overhead for 10 sec

12.Place cuvet in cuvet slot, press START

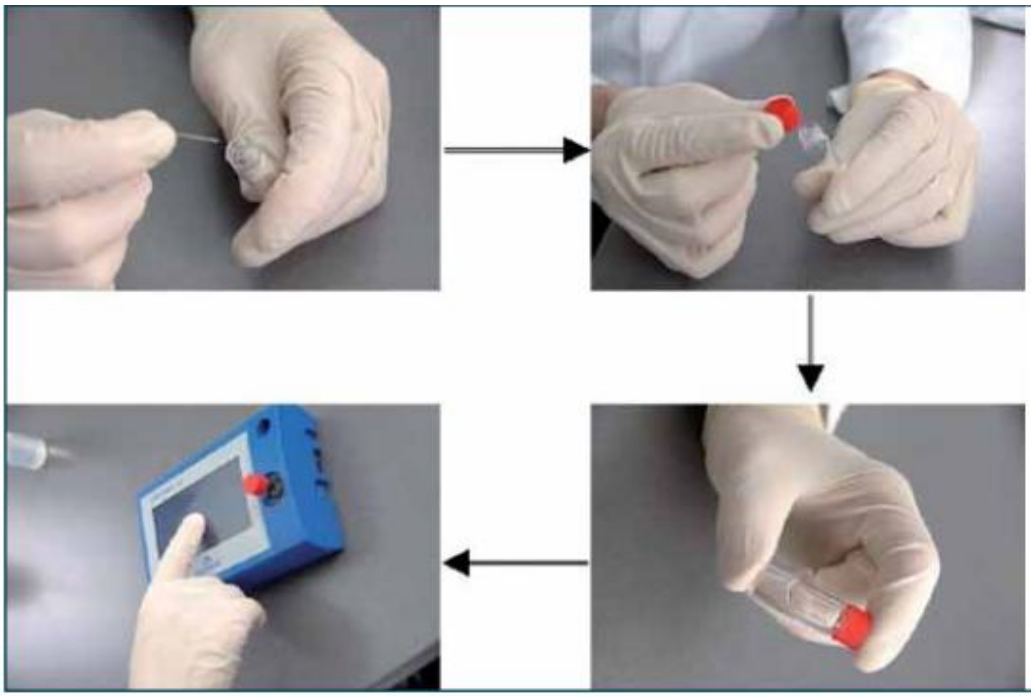
13.The result will be shown after 70 sec

At the end of 10 seconds keep the red capped cuvee in the port press the start button so that it measured the RBC AChE in 70 seconds.

Enzyme level is measured by spectrophotometric method .

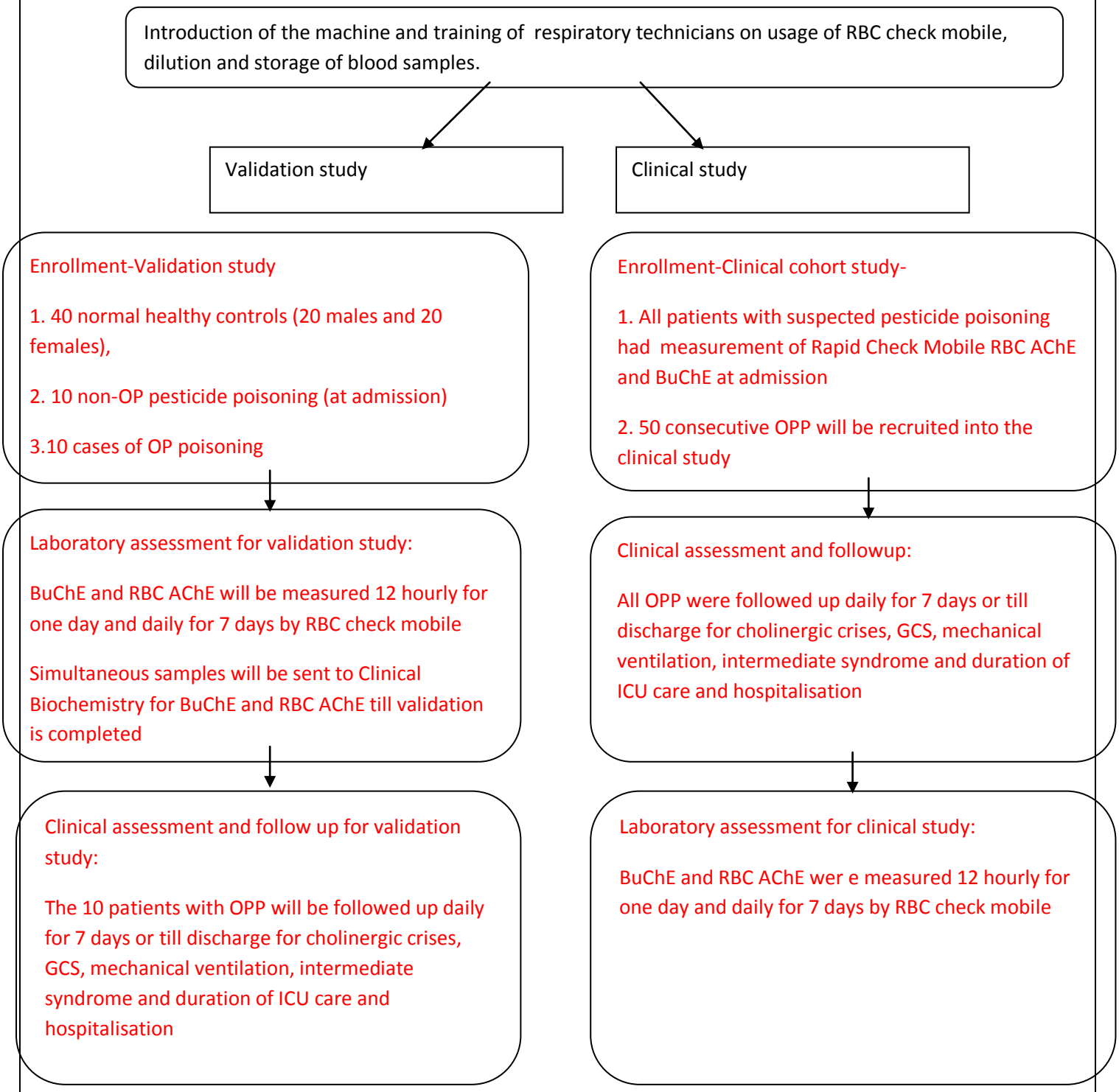
This value gets saved automatically and also will be entered in a register for each patients.

Following the RBC AChE measurement go back to the main menu and press the BChE icon. The entire process is exactly the same except for the 4 th step where instead of red cap we uses yellow cap. According to the manufacturer this machine can be used at a wide range of temperature from 10 to 50degree Celsius and the results are normalized to 37 degree celcius .Figure below shows various steps in determination of Enzyme activity.



The measurement of enzyme activity in the whole blood is by using the modified Ellman method which is different from the original Ellman by the use of BChE Inhibitor Ethopropazine, more temperature stability, and use of a different wavelength of 436nm. 436 nm reduces the background haemoglobin absorption and by using selective BChE Inhibitor, Enzymes assays can be done in whole blood and unnecessary centrifugation can be avoided.

ANNEXURE-6-DETAILED ALGORITHM OF THE STUDY



ANNEXURE 7-IRB APPROVAL

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA.

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

January 21, 2015

Dr. Aneez Joseph
PG Registrar
Department of General Medicine
Christian Medical College, Vellore 632 004

Sub: **Fluid Research Grant Project:**
Evaluation of RBC acetylcholinesterase point of care testing using ache rapid check mobile in patients with organophosphate poisoning and its correlation with clinical profile.
Dr. Aneez Joseph, PG Registrar, Dr. Anand Zachariah, Dr. J. V. Punitha, Dr. Soumya Sathyendra, Dr. Samuel George Hansdack, Dr. Ramiya. I, Medicine, Dr. Alex Reginald, Dr. K. P. P. Abhilash, Accident & Emergency Medicine, Dr. J. V. Peter, Dr. Kishore Pichamuthu, Mr. Arthur Sadhanandham, Medical ICU, Dr. Joe Fleming, Mr. Arun Jose Nallickal, Clinical Biochemistry, Dr. L. Jeyaseelan, Biostatistics, CMC, Vellore.

Ref: IRB Min No: 9203 [DIAGNOSE] dated 08.12.2014

Dear Dr. Aneez Joseph,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr. NIHAL THOMAS
SECRETARY (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Cc: Dr. Anand Zachariah, Medicine Unit - I, CMC, Vellore.

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002.
Tel : 0416 - 2284294, 2284202 Fax : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

Dr. B. J. Prashantham	MA(Counseling Psychology), MA(Theology), Dr. Min(Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Mrs. Pattabiraman	B. Sc, DSSA	Social Worker, Vellore	External, Lay Person
Dr. Denise H. Fleming	B. Sc (Hons), PhD	Honorary Professor, Clinical Pharmacology, CMC, Vellore	Internal, Scientist & Pharmacologist
Dr. Anuradha Rose	MBBS, MD	Assistant Professor, Community Health, CMC, Vellore	Internal, Clinician
Mrs. Sheela Durai	MSc Nursing	Addl. Deputy Nursing Superintendent, Professor of Nursing in Medical Surgical Nursing, CMC, Vellore	Internal, Nurse
Mr. C. Sampath	BSc, BL	Legal Expert, Vellore	External, Legal Expert
Rev. Joseph Devaraj	B. Sc, BD	Chaplainey Department, CMC, Vellore	Internal, Social Scientist
Dr. Nihal Thomas,	MD, MNAMS, DNB(Endo), FRACP(Endo) FRCP(Edin) FRCP (Glasg)	Professor & Head, Endocrinology. Additional Vice Principal (Research), Deputy Chairperson, IRB, Member Secretary (Ethics Committee), IRB, CMC, Vellore	Internal, Clinician

We approve the project to be conducted as presented.

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002.
Tel : 0416 - 2284294, 2284202 Fax : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

The Institutional Ethics Committee expects to be informed about the progress of the project, any **adverse events** occurring in the course of the project, any **amendments in the protocol and the patient information / informed consent**. On completion of the study you are expected to submit a copy of the **final report**. Respective forms can be downloaded from the following link: http://172.16.11.136/Research/IRB_Polices.html in the CMC Intranet and in the CMC website link address: http://www.cmch-vellore.edu/static/research/Index.html.

Fluid Grant Allocation:

A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an 1st Installment. The rest of the 50,000/- INR (Rupees Fifty Thousand only) each will be released at the end of the first year as 2 nd Installment following the receipt of the Interim progress/ Annual report and subsequent submission of it to the IRB.

Yours sincerely

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr. NIHAL THOMAS
MD.,MNAMS.,DNB(Endo),FRACP(Endo),FRCP(Edin),FRCP(Glasg)
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Cc: Dr. Anand Zachariah, Medicine Unit - I, CMC, Vellore.

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002.
Tel : 0416 - 2284294, 2284202 Fax : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Ortho.
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD, MNAMS, DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

November 19, 2015

Ref: IRB – A3 - 10.11.2015

Dr. Aneez Joseph,
PG Registrar,
Medicine 1,
Christian Medical College,
Vellore 632 002

Ref: IRB Min No: 9203 dated 08.12.2014

Dear Dr. Aneez Joseph,

The Institutional Review Board (Silver, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed the following amendment for the study titled "evaluation of rbc acetylcholinesterase point of care testing using ache rapid check mobile in patients with organophosphate poisoning and its correlation with clinical profile" on November 10th 2015.

1) Pregnant women and children aged 15-18 years contribute a reasonable proportion of organophosphorous poisoning that is reported in our adult causality. Due to various stressors during adolescence and pregnancy incidence of poisoning among these population is high. By including this population , it enhances the applicability of this study to different population groups

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on November 10th 2015 at 12.45 p.m in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Affiliation
Dr. Nihal Thomas	MD, MNAMS, DNB(Endo), FRACP (Endo) FRCP(Edin) FRCP (Glasg)	Professor & Head, Endocrinology. Additional Vice Principal (Research), Deputy Chairperson(Research Chairperson), Member Secretary (Ethics Committee), IRB. CMC, Vellore	Internal, Clinician
Dr. Vivek Mathew	MD (Gen. Med.) DM (Neuro) Dip. NB (Neuro)	Professor, Neurology, CMC, Vellore	Internal, Clinician
Dr. Mathew Joseph	MBBS, MCH	Professor, Neurosurgery, CMC, Vellore	Internal, Clinician

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Ortho.
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD, MNAMS, DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Ms. Grace Rebecca	MSc (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Dr. Anuradha Rose	MBBS, MD, MHSC (Bioethics)	Associate Professor, Community Health CMC, Vellore	Internal, Clinician

We approve the above amendment as presented.

Yours sincerely,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr. NIHAL THOMAS
MD,MNAMS,DNB(Endo),FRACP(Endo),FRCP(Edin),FRCP(Glasg)
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

IRB Min No: 9200 dated 08.12.2014

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

ANNEXURE-8-DATA SHEET

sl.no	Machine ACHE	Lab ACHE	machine Bche	lab bche					
1	46.3	44.94	3175.4	6679	NON OP PESTICIDES				
2	39.8	35.99	2231.6	5496	sl.no	Machine ACHE	Lab Ache	Machine Bche	lab Bche
3	44.4	43.57	3360	7677	1	36.3	49.8	2885.9	7594
4	32.2	35.75	2952.6	7126	2	40.4	38.64	2295.3	6667
5	37.1	34.434	3115.7	7102	3	39	33.0238982	1872.9	5581
6	38.6	38.058	3321.2	7079	4	45.4	29.1129905	2747.1	
7	42.7	37.56	3423.6	8081	5	40.8	24.956	2934.4	
8	50.6	44.7	2495.4	6784	6	40.3	66.01	2608.9	
9	36.8	32.31	2835.8	6094	7	34.5	46.57	2774.5	3930
10	40.4	42.098	3214.5	6964	8	37.3	41.07	2841.1	6947
11	33.8	33.35	3044.6	6225	9	35.4	58.9	2154	5827
12	37.8	37.717	2556.6	6072	10	32.1	54.35		6987
13	41	40.956	2532.4	5076	11	39.4	35.193	2934.2	5801
14	38.5	33.83	3724.4	8744	12	26.2	26.16	2426.9	5828
15	38	37.5263469	3436.3	7483					
16	28.4	34.58911	3283.7	8952					
17	26.9	38.5464636	2682.5	6493					
18	45.4	51.2569053	3185.4	7333					
19	34.8	43.425319	2923.7	6551					
20	44.4	50.7634	2705.1	6364					
21	44.1	33.9810113	1434.7						
22	37.7	28.148662	2589						
23	50.4	38.6415845	3073.3						
24	40.9	34.42	2252	4946					
25	41.2	36.7	3500.7	8234					
26	43.9	43.09	3103.9	7211					
27	30	31.872204	1680.6	8965					
28	36.1	38.1068266	3416.3	8799					
29	43.2	43.2603194	3315.2	8765					
30	37.1	44.3514164	5289.8	8023					
31	36.2	48.5664849	2884.4	6708					
32	30.6	39.3314076	3583.5	10116					
33	25	42.9028407	1501.3	8081					
34	25.6	32.5251544	3037	7718					
35	34.4	37.706648	2847.4						
36	43.4	37.115452	2327						
37	39.2	21.6486844	2435.5						
38	28.4	38.92142	2615.4	10115					
39	38	31.5119523	2269.4						
40	38.9	26.1868524	2208.6						
41	38.6	31.2884186	3162						
42	42.6	63.6472759	3436.5						
43	40	22.414436	2822.2						

IDNO	CASE	AGE	SEX	EDU	PROF	COMPID	COMPN	COMPNAME	COMPPCT	COMPTYP	COMPQUA	COMPPYRE	PYRENAME	FMCT	FMCT1	CMCT	CMCT1	TIMEINT	TIMEINT	TOUT	TOUTSH	TOUTEN
1	1	20	2	2	1	1	3	dichlorvos	76	1	20	0		21	15	21	15	1.0	1.0	0	.	.
2	1	34	2	1	1	1	3	phorate	10	2	10	0		21	45	9	40	1.0	13.0	1	0	1
3	2	26	1	2	2	0	.		.	.	.	.		7	30	8	40	2.5	3.6	1	0	0
4	1	34	1	2	2	1	3	quinalphos	25	2	60	0		20	40	20	40	1.0	1.0	0	.	.
5	1	24	2	1	1	1	2	methylparathion	2	1	100	0		21	15	21	15	3.0	3.0	0	.	.
6	1	20	2	2	1	1	3	prefoenofos	40	3	20	1	cypermethrin	21	50	21	50	2.0	2.0	0	.	.
7	1	26	1	2	1	1	3	profenofos	40	3	50	1	cypermethrin	21	50	21	50	2.0	2.0	0	.	.
8	1	15	2	2	1	1	3	monocrotophos	36	1	20	0		21	0	22	15	5.0	6.2	1	0	0
9	1	27	1	2	2	1	3	dimethoate	35	1	50	0		23	50	23	50	5.0	5.0	0	.	.
10	1	27	1	2	2	1	3	monocrotophos	36	1	20	0		9	0	21	45	0.5	13.2	1	0	0
11	1	17	2	1	.	1	3	monocrotophos	36	1	20	0		8	0	12	20	1.0	5.3	1	0	1
12	1	24	2	1	1	1	3	dimethoate	35	1	75	0		18	0	20	50	1.0	4.0	1	0	1
13	1	19	2	2	3	1	3	quinalphos	25	2	200	0		12	0	21	30	1.0	10.5	1	0	1
14	1	25	2	1	1	1	2	profenofos	50	3	100	0		6	35	6	35	10.5	10.5	0	.	.
15	1	17	2	1	1	1	3	profenofos	40	3	250	1	cypermethrin	8	0	18	40	1.0	11.6	1	0	0
16	1	19	1	1	1	1	2	dimethoate	30	1	100	0		12	0	18	20	0.5	7.0	1	0	0
17	1	20	2	1	1	1	3	monocrotophos	36	1	50	0		20	30	11	0	1.0	15.5	1	0	1
18	1	19	1	1	1	1	3	dimethoate	30	1	75	0		16	0	15	0	4.0	29.0	1	0	0
19	2	46	1	1	1	0	.		.	.	.	.		23	0	12	10	4.0	5.0	1	0	0
20	1	55	1	1	1	1	2	traizophos	35	2	25	1	deltamethrin	15	0	15	30	1.0	1.5	1	0	0
21	1	45	1	1	1	1	3	chlorpyrifos	40	2	15	1	cypermethrin	21	30	0	0	0.5	3.0	1	0	0
22	1	17	2	2	1	1	3	chlorpyrifos	20	2	150	0		11	30	16	30	1.5	5.5	1	0	0
23	1	45	2	1	1	1	3	triazophos	40	2	100	0		11	.	22	0	1.0	13.0	1	0	0
24	1	23	1	2	.	1	3	phorate	10	2	50	0		19	30	23	0	1.0	4.5	1	0	0
25	2	22	1	2	1	0	.		.	.	.	.		22	0	2	.	2.0	2.0	0	.	.
26	1	28	1	1	1	1	3	monocrotophos	30	1	10	0		17	30	17	30	3.0	3.0	0	.	.
27	2	64	2	1	1	0	.		.	.	.	.		13	30	14	45	1.5	2.8	1	0	0
28	1	24	2	2	1	1	3	triazophos	40	2	50	0		10	30	13	30	1.5	4.5	1	0	0
29	1	33	2	2	1	1	3	malathion	5	1	100	0		9	30	9	30	1.5	1.5	0	.	.
30	1	37	1	1	2	1	3	triazophos	35	2	250	1	deltamethrin	17	0	1	0	1.5	7.5	1	0	0
31	1	21	2	2	.	1	2	profenofos	40	3	30	1	cypermethrin4	9	30	1	0	1.0	4.5	1	0	0
32	2	35	2	1	1	0	.		.	.	.	.		19	0	20	.	2.0	3.0	1	0	0
33	1	49	1	2	1	1	3	malathion	5	1	50	0		11	30	14	0	0.5	3.0	1	0	0
34	1	25	2	2	.	1	3	chlorpyrifos	20	2	100	0		13	.	16	30	0.5	4.0	1	0	1
35	1	38	1	1	1	1	3	ethion	40	4	100	1	cypermethrin	10	.	13	.	1.0	5.0	1	0	0
36	1	25	2	2	1	1	3	chlorpyrifos	20	2	40	0		10	30	21	10	1.0	3.2	1	0	0
37	1	30	2	2	1	1	2	triazophos	35	2	100	0		17	30	19	45	0.5	2.8	1	0	0
38	1	53	2	1	1	1	3	Dichorphos	76	1	25	0		10	30	13	30	1.0	3.7	1	0	1
39	1	30	2	1	1	1	3	phorate	10	2	10	0		14	30	17	30	2.0	5.0	1	0	0
40	1	24	2	1	1	1	3	chlorpyrifos	20	2	.	0		.	.	13	0	.	.	1	0	0
41	1	31	1	1	2	1	2	dichlorvos	76	1	30	1	cyhlothrin	12	.	20	.	1.0	9.0	1	0	0
42	1	1	1	1	1	1	2	phorate	10	2	.	0		16	0	18	30	1.0	3.5	1	0	0
43	1	27	2	1	2	1	2	phorate	10	2	100	0		19	0	21	.	2.0	4.0	1	0	0
44	1	27	2	2	1	1	2	triazophos	35	2	100	1	deltamethrin	20	0	20	30	1.0	1.5	1	0	0
45	1	65	1	1	1	1	1	chorpyrifos	20	2	100	0		5	30	9	15	4.5	8.3	1	0	1
46	1	60	1	1	1	1	1	triazophos	.	2	100	1	deltamethrin	14	30	6	30	1.0	15.0	1	0	0
47	1	25	1	1	1	1	2	chlorpyrifos	50	2	300	1	cypermethrin	5	0	6	0	7.0	8.0	1	0	0
48	2	35	1	1	1	0	.		.	.	.	.		.	.	21	0	0.5	0.5	0	.	.
49	1	55	1	1	1	1	1	dimethoate	30	1	50	0		10	30	19	0	0.5	9.0	1	0	0
50	1	29	1	1	1	1	3	triazophos	35	2	100	0		20	.	23	30	0.5	4.0	1	0	0
51	1	55	1	1	1	1	3	chlorpyrifos	50	2	100	1	cypermethrin	23	45	16	0	2.3	18.5	1	0	0
52	1	23	2	1	2	1	3	Triazophos	40	2	100	0		11	30	6	0	2.0	8.5	1	0	0
53	1	45	1	1	1	1	2	monocrotophos	36	1	400	0		21	45	23	.	1.0	2.3	1	0	0
54	1	19	1	2	1	1	3	profenofos	40	3	15	1	cypermethrin	11	30	13	0	0.5	2.0	0	.	.
55	1	22	1	1	2	1	2	Phorate	10	2	50	0		13	0	20	5	1.0	8.0	1	0	1
56	1	18	1	1	1	1	3	profenofos	40	3	50	1	cypermethrin	1	0	4	30	5.5	9.0	1	0	0
57	1	24	1	1	1	1	3	profenofos	50	3	30	0		14	30	15	30	0.5	2.0	1	0	0
58	1	23	1	1	1	1	1	monocrotophos	36	1	20	0		12	30	13	30	1.0	2.0	1	0	0
59	1	20	1	2	2	1	1	Chlorpyrifos	.	2	50	1	cyermethrin	20	0	21	.	0.5	1.5	1	0	0

TOUTG	TOUTA	ATRDO	TOUTP	PAMDO	TOUTIN	TOXISA	TOXILA	TOXIUR	TOXIDE	TOXIVO	TOXISZ	TOXIBR	TOXIFA	TOXIAL	ADMGC	ADMPU	ADMPR	ADMBP	ADMBP	ADMRR	ADMSA	ADMGR	PRESV
.	.	.	.	.	.	1	0	0	0	1	0	0	0	0	15	3	102	120	80	20	100	204	1
1	1	99	1	99	0	0	0	0	0	1	0	1	1	0	15	3	99	120	80	34	100	222	1
0	0	.	0	.	0	1	0	1	1	1	0	0	0	0	15	1	100	170	90	20	100	139	1
.	.	.	.	.	.	1	0	0	0	0	0	1	0	1	3	1	109	100	60	.	80	150	0
.	.	.	.	.	.	1	0	0	0	1	0	0	0	0	15	3	60	110	60	20	90	162	1
.	.	.	.	.	.	1	0	0	0	1	0	0	0	0	15	3	84	120	80	24	97	130	1
.	.	.	.	.	.	1	0	0	0	1	0	0	0	0	15	1	86	120	70	24	99	130	1
0	1	99	0	.	1	1	0	1	0	1	1	1	0	0	7T	1	100	100	60	.	100	269	1
.	.	.	.	.	.	1	0	0	0	1	0	0	0	0	15	3	80	94	60	24	97	170	1
1	1	99	0	.	1	1	0	1	0	0	1	0	0	0	10T	1	86	100	60	.	96	143	1
1	1	99	0	.	1	1	0	0	0	1	0	0	0	1	3T	3	102	110	80	.	100	284	1
1	1	4	0	.	0	1	1	0	0	1	0	1	0	1	7	1	125	100	60	8	80	151	1
1	1	8	1	99	0	1	0	1	0	1	0	0	0	0	15	3	106	100	70	20	97	138	1
.	.	.	.	.	.	1	0	0	0	1	0	1	1	0	15	3	112	100	60	20	97	158	1
1	1	99	1	99	0	1	1	1	0	1	0	1	0	0	15	2	140	100	70	26	98	348	1
1	1	12	0	.	0	1	1	0	0	1	0	0	0	1	14	2	126	120	80	16	97	175	1
1	1	99	0	.	1	1	1	1	0	1	1	0	1	1	5T	1	84	100	60	.	99	200	0
1	0	.	0	.	0	1	0	0	0	1	0	0	1	0	3	1	152	90	60	.	70	193	1
1	1	2	1	5	0	1	0	0	0	1	0	0	0	0	15	3	108	120	80	20	99	148	1
1	0	.	0	.	0	1	0	0	0	1	0	0	0	0	15	1	130	120	80	22	86	177	1
1	1	99	0	.	0	1	0	0	0	0	0	0	0	0	15	3	110	110	80	20	98	95	1
1	1	20	0	.	0	0	0	0	0	1	0	0	0	1	15	2	78	110	80	21	96	98	1
1	0	.	0	.	0	0	0	0	1	1	0	0	0	1	12	1	110	110	60	24	96	216	1
0	1	99	1	99	0	1	1	0	0	1	0	0	0	0	15	3	104	110	70	24	98	92	1
.	.	.	.	.	.	1	0	0	0	1	0	1	0	1	3	3	84	140	90	.	64	192	1
.	.	.	.	.	.	0	0	0	0	0	0	0	0	0	15	3	100	100	70	12	100	197	0
1	1	15	0	.	0	0	0	1	0	1	0	0	0	0	14	3	102	90	60	22	98	159	1
1	1	6	1	2	0	1	1	1	1	1	0	0	0	1	14	3	100	100	70	26	98	229	1
.	.	.	.	.	.	1	0	0	0	1	0	0	0	0	15	3	75	100	70	24	95	94	1
1	0	.	0	.	0	1	0	1	0	1	0	1	0	1	15	1	112	100	60	44	901	194	0
1	0	.	0	.	0	0	0	0	0	1	1	0	0	1	14	3	134	90	60	20	99	197	1
1	1	99	0	.	0	1	1	1	0	1	0	0	0	0	15	1	128	110	70	28	78	239	1
1	1	99	0	.	0	0	0	0	0	1	0	0	0	0	15	3	110	160	90	20	98	135	1
0	1	30	0	.	0	1	0	0	1	0	1	0	0	1	14	3	108	120	80	24	98	93	1
1	1	99	1	99	0	0	0	0	1	0	0	0	0	1	3	1	128	110	80	36	92	278	0
1	1	3	0	.	0	0	0	0	0	1	0	0	0	0	15	3	118	110	80	28	100	82	1
1	1	99	0	.	0	0	0	1	1	1	0	0	0	1	8	3	103	100	70	12	94	121	0
1	1	99	0	.	0	1	0	0	0	1	0	1	0	1	12	3	126	110	70	22	100	293	1
1	1	20	1	1	0	0	0	0	0	1	0	0	0	0	15	3	148	100	80	22	94	110	1
1	0	.	0	.	1	1	1	0	1	1	0	1	0	1	10	1	140	100	60	.	58	197	1
1	1	99	1	99	0	0	0	0	0	0	1	0	0	1	12	3	116	140	90	24	98	130	0
1	1	99	0	.	0	1	1	1	1	1	1	0	0	1	13	3	102	140	80	18	98	158	1
1	1	99	1	99	0	1	1	1	1	1	0	0	0	1	9	1	96	126	80	44	93	132	0
1	1	3	0	.	0	0	0	0	0	0	0	1	0	1	3	1	114	100	70	36	70	178	0
1	1	10	1	99	0	0	0	0	1	1	0	0	0	0	15	3	116	120	90	26	98	314	1
1	0	.	0	.	0	1	0	1	1	0	0	0	0	1	14	1	154	150	90	40	92	153	1
1	1	13	0	.	0	0	0	1	0	0	0	0	0	0	14	3	130	140	80	22	96	158	0
.	.	.	.	.	.	0	0	0	0	0	0	0	0	0	14	3	66	110	70	22	96	164	1
1	0	.	0	.	1	0	0	0	0	0	0	0	0	1	10t	1	120	180	90	.	100	137	0
1	0	.	0	.	0	0	0	0	0	1	0	0	0	1	10	3	126	140	80	26	96	250	1
1	1	5	0	.	0	0	0	0	0	1	1	0	0	1	3	2	68	100	70	22	86	202	0
0	1	5	1	99	0	0	0	0	0	1	0	0	0	1	3	3	68	100	70	22	86	202	1
1	1	4	0	.	0	0	0	0	1	0	0	0	0	0	7	3	146	180	90	32	88	86	0
.	.	.	.	.	.	0	0	0	0	1	0	0	0	0	15	3	134	100	60	20	99	101	0
1	1	99	0	.	1	1	0	0	0	0	0	1	0	1	10t	1	100	110	70	.	98	111	0
1	0	40	0	.	0	0	0	1	1	0	0	0	0	1	7	3	130	160	90	32	94	208	0
1	0	.	0	.	0	1	1	0	0	1	0	0	0	0	14	1	86	110	80	20	96	106	1
1	1	12	1	99	0	1	1	1	0	1	0	0	0	1	6	1	68	140	90	26	96	230	1
1	1	99	1	99	0	0	1	0	0	1	0	0	0	0	15	3	124	130	80	18	98	228	1

PRESDI	PRESAE	PRESSA	PRESSV	PRESUI	PRESDI	PRESBF	PRESSZ	PRESBL	PRESGI	PRESAL	PRESAC	PRESAS	SIGNDI	SIGNPU	SIGNCR	SIGNSA	SIGNFR	SIGNFA	SIGNSB	SIGNPB	SIGNAB	SIGNFE	SEVERI
0	1	1	1	0	0	0	0	0	0	0	0	0	1	3	0	1	0	0	41	0	0	0	2
0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	1	21	0	0	1	3
1	0	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1	1	0	.	0	0	0	3
0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	1	1	0	0	.	1	0	0	4
0	0	1	0	0	0	0	0	0	0	0	0	0	0	3	0	1	0	0	5	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	.	0	0	0	2
0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	24	0	0	0	2
0	0	1	0	1	1	1	1	0	0	1	0	0	0	1	1	1	0	0	.	0	0	0	4
0	0	1	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	.	0	0	0	2
0	0	1	1	1	0	0	1	0	0	0	0	0	0	1	0	0	0	1	.	0	0	0	4
0	0	1	0	0	1	0	0	0	0	1	0	0	0	3	0	1	0	0	.	0	0	0	4
0	0	1	1	0	0	1	0	0	0	1	1	0	1	1	1	1	0	0	.	0	0	0	4
0	0	1	0	0	0	0	0	0	0	0	0	0	0	3	0	1	0	0	24	0	0	0	1
0	0	1	0	0	0	0	0	0	0	0	1	0	0	2	1	1	0	1	17	0	0	0	4
0	0	1	1	0	1	1	0	0	0	0	1	0	1	7	1	1	1	0	.	0	0	0	4
0	0	1	1	0	0	0	0	0	0	1	1	0	1	6	0	1	0	0	15	0	0	0	3
0	0	0	0	0	1	1	1	0	0	1	0	0	0	1	0	0	0	1	.	0	0	0	4
0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	1	1	0	1	.	1	0	0	4
0	0	1	1	0	0	0	1	0	0	0	0	0	1	4	1	1	1	0	.	0	0	0	4
0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	20	0	0	0	2
0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0	0	3
0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	30	0	0	0	2
0	0	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	.	0	0	1	3
0	0	1	0	0	0	0	0	0	0	0	0	0	0	5	0	1	0	0	30	0	0	0	2
0	0	1	0	0	1	1	0	0	0	1	0	0	0	3	1	1	1	0	.	1	0	0	4
0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	35	0	0	0	1
0	0	0	0	1	1	0	0	0	0	1	0	0	0	2	0	0	0	0	14	0	0	0	2
0	0	1	0	1	1	0	0	0	0	0	0	0	0	5	1	1	0	0	13	0	0	0	3
0	0	1	0	0	0	0	0	0	0	0	0	0	1	4	0	1	0	0	20	0	0	0	2
0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	1	.	0	0	0	4
0	0	1	0	0	1	0	0	0	0	1	0	0	0	3	1	1	0	0	32	0	0	0	2
1	0	1	0	1	0	0	0	0	0	1	0	0	0	1	1	1	1	1	.	1	0	0	4
0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	30	0	0	0	2
0	0	0	0	0	1	0	0	0	0	1	1	0	0	4	0	0	0	0	.	0	0	0	3
0	0	0	0	0	1	1	0	0	0	1	1	0	0	1	1	0	1	1	.	1	0	0	4
0	0	0	0	0	0	0	1	0	1	0	0	0	0	4	0	0	0	0	30	0	0	1	4
0	0	1	0	0	0	0	0	0	0	1	0	0	0	4	1	1	0	0	.	0	0	0	4
0	0	1	0	0	1	0	0	0	0	1	1	0	0	2	1	1	1	0	.	0	0	1	3
0	0	0	0	0	0	0	0	0	0	0	1	0	0	3	0	0	0	0	10	0	0	0	2
1	0	1	0	0	1	1	0	0	0	1	0	0	0	1	1	0	0	0	.	0	0	0	4
0	0	0	0	0	1	0	0	0	0	1	1	0	0	3	0	0	0	0	.	9	9	9	3
1	0	1	0	1	1	1	0	0	0	1	1	0	0	4	1	1	1	1	10	1	0	0	4
0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	1	1	1	1	3	1	0	0	4
0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	1	1	0	0	.	1	0	0	4
0	0	0	0	0	0	1	0	0	0	0	0	0	0	3	0	0	0	0	.	0	0	0	4
1	1	1	0	0	0	1	0	0	0	1	0	0	0	1	1	1	1	0	.	0	0	0	4
0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	30	0	0	0	2
1	0	1	0	0	1	0	0	0	0	1	1	0	0	3	0	1	0	0	.	0	0	0	3
0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	.	1	0	0	4
0	0	0	0	1	0	0	0	0	0	1	0	0	0	4	0	0	0	0	20	0	0	0	3
0	0	0	0	0	1	0	1	0	0	1	0	0	0	3	1	1	0	1	.	1	0	0	4
0	0	0	0	0	1	1	1	0	0	1	0	0	0	3	1	0	0	0	.	0	0	0	4
0	0	0	1	0	0	1	0	0	1	1	0	0	1	3	1	0	0	0	.	1	0	0	4
0	0	0	0	0	0	0	0	0	0	0	0	1	0	4	0	0	0	0	18	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	0	.	0	0	0	4
0	0	0	0	0	1	1	0	0	0	1	0	0	0	3	1	0	0	1	.	1	0	0	4
0	1	1	1	0	0	0	0	0	0	1	0	1	1	1	0	1	0	0	20	0	0	0	2
0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	1	1	1	0	.	1	0	0	4
0	0	1	0	1	0	0	0	0	0	0	0	0	1	3	0	1	1	0	20	0	0	0	2

HR0	SBP0	DBP0	GCS0	GCI0	SHOULI	ELBOW	HAND0	HIP0	NHF0	RESPW	VENTM	TIDAL0	PS0	PEEP0	SBC0	CHOLC	ATRBO	ATRINF	ATRTO	SED0	HR2	SBP2	DBP2	GCS2	GCSI2
102	120	80	15		5	5	1	5	50	0	1	.	.	.	41	1	0.0	0.0	0.0	0	90	120	80	15	
99	120	80	15		5	5	1	5	26	0	1	.	.	.	21	1	4.8	111.0	115.8	0	100	130	90	15	
110	130	70	10	T	4	4	1	4	4	1	3	450	12	6	.	1	39.0	24.0	63.0	0	90	120	60	15	
106	100	60	5	T	3	3	2	3	.	1	3	550	15	6	.	1	3.6	31.0	34.6	1	90	130	80	2	T
60	110	60	15		4	4	1	4	2	0	1	.	.	.	5	1	2.4	20.0	22.4	0	100	110	60	2	T
84	120	80	15		5	5	1	5	.	0	1	.	.	.	.	1	0.0	0.0	0.0	0	98	90	60	15	
86	120	70	15		5	5	1	5	40	0	1	.	.	.	24	1	1.8	18.0	19.8	0	80	120	60	15	
100	120	70	7	T	2	2	2	2	.	1	3	320	15	5	.	1	17.2	73.5	90.7	1	140	130	90	9	T
80	94	60	15		5	5	1	5	40	0	1	.	.	.	20	1	1.8	0.0	1.8	0	140	110	70	15	
86	100	60	10	T	4	4	1	4	3	1	3	400	15	5	.	1	24.4	114.0	138.5	1	101	130	80	7	T
102	110	80	3	T	2	2	2	2	.	1	3	350	15	7	.	1	5.0	23.0	28.0	1	97	110	60	7	T
125	100	60	7		3	3	1	3	5	1	3	300	15	10	.	1	6.0	24.3	30.3	1	108	100	50	10	T
106	100	70	15		5	5	1	5	60	0	1	.	.	.	24	1	4.8	0.0	4.8	0	100	100	70	15	
112	100	60	15		3	3	2	3	10	1	3	300	15	6	17	1	39.3	98.0	137.3	0	118	100	60	10	T
140	100	70	15		4	4	1	4	2	1	3	300	15	5	.	1	2.4	55.5	57.9	0	90	120	70	10	T
126	100	60	14		5	5	1	5	16	0	1	.	.	.	15	1	1.2	8.5	9.7	0	106	100	60	15	
84	100	60	5	T	2	2	2	2	.	1	3	360	15	7	.	1	252.0	426.5	678.5	1	87	110	60	2	T
152	90	60	3		1	1	2	1	10	1	3	400	13	5	.	1	124.0	203.0	327.0	1	100	120	70	10	T
108	120	80	8	t	3	3	2	3	.	1	3	400	15	5	.	1	18.6	39.0	57.6	1	108	150	90	9	t
130	120	80	15		5	5	1	5	25	0	0	.	.	.	25	1	0.6	28.0	28.6	0	118	120	80	15	
100	110	80	15		5	5	1	5	15	0	1	.	.	.	20	1	0.0	28.0	28.0	0	102	110	70	14	
122	100	70	15		5	5	1	5	20	0	1	0	.	.	30	0	16.0	0.0	16.0	0	126	100	70	15	
102	110	70	12		4	4	1	4	10	0	1	.	.	.	.	1	0.0	9.0	9.0	0	100	130	70	14	
104	110	70	15		5	5	2	5	40	0	1	.	.	.	30	0	0.0	0.0	0.0	0	102	100	70	15	
84	140	90	3	t	1	1	2	1	.	0	3	400	15	8	.	1	1.2	42.0	43.2	1	100	130	80	8	t
100	100	60	15		5	5	1	5	30	0	1	0	.	.	35	0	0.0	0.0	0.0	0	92	100	60	15	
102	90	60	14		5	5	1	5	99	0	1	.	.	.	14	1	1.8	23.0	33.8	0	.	.	.	.	
100	100	70	14		5	5	1	5	15	0	1	.	.	.	13	1	39.6	50.0	89.6	0	128	104	70	14	
75	100	70	15		5	5	1	5	45	0	1	.	.	.	20	1	1.0	12.0	13.0	0	87	100	70	15	
110	100	60	15		3	3	2	3	1	1	3	400	15	5	.	1	16.0	42.0	58.0	1	100	130	80	9	t
134	90	60	14		5	5	1	5	1	0	1	.	.	.	32	1	18.0	15.5	33.5	0	132	100	70	15	
92	110	70	2	t	3	3	1	3	.	1	3	400	15	7	.	1	20.0	35.5	55.5	0	98	110	70	10	t
100	160	90	15		5	5	1	5	60	0	1	.	.	.	30	1	0.0	53.0	53.0	0	102	140	90	15	
100	120	70	14		4	4	1	4	20	0	1	.	.	.	19	0	0.0	0.0	0.0	0	108	120	70	15	
128	160	90	3		3	3	2	3	.	1	1	400	15	8	.	1	78.0	313.5	391.5	1	104	126	58	8	t
100	100	80	15		4	4	2	4	60	0	1	.	.	.	30	0	1.8	0.0	1.8	0	82	100	70	6	t
100	100	70	10	t	4	4	1	4	.	1	3	400	10	6	.	1	3.6	190.0	193.6	1	102	120	70	6	t
100	100	70	12		4	4	1	4	.	0	.	.	.	.	99	1	21.0	0.0	21.0	0	98	100	70	15	
106	90	60	15		4	4	1	4	10	0	0	0	.	.	10	0	14.0	8.0	34.0	0	102	100	60	15	
120	100	70	3	t	3	3	2	3	.	3	3	400	10	7	.	1	45.0	46.0	91.0	0	108	100	70	10	t
110	130	80	12		3	3	1	3	.	1	1	.	.	.	.	0	0.0	8.0	8.0	0	100	130	80	15	
102	140	80	13		3	3	2	3	10	3	3	400	15	7	10	1	458.8	100.0	558.4	0	100	140	80	10	t
110	110	70	2	t	3	3	2	3	.	1	3	400	15	5	3	1	60.0	100.0	160.0	1	102	110	70	9	t
114	100	70	2	t	3	3	2	3	.	1	3	400	15	5	.	1	45.0	207.0	253.8	1	102	100	70	10	t
110	110	80	10	t	4	4	2	4	30	0	3	400	17	12	.	0	10.0	10.0	20.0	1	98	110	80	10	t
130	130	90	10	t	3	3	2	3	5	1	3	400	15	7	.	1	3.0	29.0	32.0	1	88	120	80	6	t
138	120	70	14		4	4	1	4	20	0	1	.	.	.	30	0	0.0	3.3	23.3	0	98	120	70	14	
100	100	70	11		3	3	2	3	.	0	1	.	.	.	.	1	10.0	25.0	35.0	0	98	120	80	14	
88	100	70	10	t	3	3	2	3	.	1	3	400	15	5	.	1	0.0	40.0	40.0	1	86	100	70	4	t
120	140	80	11		4	4	1	4	30	0	1	.	.	.	20	0	0.0	8.0	8.0	0	94	130	80	14	
95	120	80	2	t	2	2	2	2	.	0	3	400	15	7	.	1	45.0	227.0	275.0	1	96	140	80	7	t
96	120	80	2	t	3	3	2	3	.	1	3	400	15	5	.	1	45.0	183.0	228.0	0	96	140	80	7	t
94	180	90	7	t	3	3	2	3	.	1	3	400	15	7	.	1	1.0	60.0	63.0	1	98	120	80	10	t
134	100	60	15		5	5	1	5	20	0	1	.	.	.	18	0	0.0	30.0	30.0	0	100	100	60	15	
100	100	70	10	t	4	5	1	5	.	1	3	400	12	5	.	1	24.0	48.0	72.0	1	104	110	70	10	t
114	130	80	10	t	4	4	1	4	.	1	3	400	15	7	.	1	24.0	28.5	52.5	1	104	130	80	8	T
86	136	88	15		5	5	1	5	20	0	1	.	.	.	20	0	1.8	13.0	14.8	0	88	120	80	14	
68	120	80	2	t	3	3	2	3	.	1	3	400	12	5	.	1	20.0	92.0	124.0	1	84	120	70	10	t
120	120	80	15		5	5	1	5	45	0	1	.	.	.	20	0	0.6	0.5	1.1	0	98	120	80	15	

SHOULD2	ELBOW2	HAND2	HIP2	NHF2	RESPWEAK2	VENTMODE2	SBC2	CHOLCRIS2	ATRBOLUS2	ATRTOTAL2	HR3	SBP3	DBP3	GCS3	GCSI3	SHOULI	ELBOW	HAND3	HIP3	NHF3	RESPW
5	5	1	5	50	0	1	29	0	0.0	0.0	90	120	80	15		5	5	1	5	50	0
4	4	1	4	5	0	1	25	1	0.0	66.0	80	100	70	10	T	3	3	2	3	5	1
5	5	1	5	25	0	1	27	0	0.0	0.0	80	140	80	15		5	5	1	5	35	0
1	1	2	1	99	0	3	99	1	0.6	256.6	110	120	70	6	T	2	2	2	2	0	1
1	1	2	1	99	1	3	99	1	0.6	183.6	110	110	70	9	T	3	3	2	3	0	1
5	5	1	5	20	0	1	13	0	0.6	0.6	90	100	60	15		5	5	1	5	20	0
5	5	1	5	60	0	1	50	0	0.0	4.0	80	120	70	15		5	5	1	5	60	0
2	2	2	2	99	1	3	99	1	0.0	141.0	90	120	70	10	T	3	3	2	3	0	1
5	5	1	5	40	0	1	21	1	2.0	22.5	122	110	70	15		5	5	1	5	40	0
4	4	1	4	0	1	3	99	1	0.0	645.0	100	150	80	10	T	4	4	1	4	2	1
3	3	1	3	0	1	3	99	1	0.0	23.0	100	120	80	15		4+	4+	1	4+	40	0
3	3	1	3	0	1	3	99	1	0.0	47.7	95	110	70	10	T	4	4	1	4	1	1
5	5	1	5	60	0	1	20	1	4.2	41.5	100	100	70	15		5	5	1	5	30	0
3	3	2	3	4	1	3	99	1	0.0	53.0	100	100	60	10	T	3	3	2	3	0	1
4	4	1	4	0	1	3	99	1	0.0	45.0	105	120	70	10	T	3	3	1	3	0	1
5	5	1	5	60	0	1	36	1	0.0	19.5	80	100	60	15		5	5	1	5	60	0
1	1	2	1	99	1	3	99	1	0.0	389.0	96	130	70	2	T	1	1	2	1	99	1
3	3	2	3	0	1	3	99	1	0.0	171.0	100	130	70	10	T	3	3	2	3	1	1
1	1	2	1	99	1	3	99	0	0.0	126.0	90	140	80	9	t	2	2	2	2	99	1
5	5	1	5	30	0	1	30	0	0.0	73.5	96	120	80	15		5	5	1	5	30	0
5	5	1	5	20	0	1	15	0	0.0	65.0	118	110	70	15		5	5	1	5	20	0
5	5	1	5	20	0	1	30	0	0.0	0.0	130	120	70	14		5	5	1	5	10	0
5	5	1	5	10	0	1	10	1	0.0	57.0	104	120	70	15		5	5	1	5	10	0
5	5	1	5	40	0	1	25	0	1.8	1.8	100	100	70	15		5	5	1	5	40	0
2	2	2	2	99	0	3	99	1	0.0	110.0	110	120	80	8	t	3	3	2	3	5	1
5	5	1	5	40	0	0	40	0	0.0	0.0	90	110	60	15		5	5	1	5	60	0
		.		.	.	.	.	.	.	.	.	.	.	.				.		.	.
5	5	1	5	40	0	1	20	0	0.0	19.0	94	100	70	15		5	5	1	5	40	0
5	5	1	5	25	0	1	20	0	0.0	8.0	86	110	70	15		5	5	1	5	25	.
3	3	2	3	0	1	3	99	1	0.0	132.0	96	130	90	10	t	3	3	2	3	5	1
5	5	1	5	30	0	1	24	0	0.0	1.3	116	100	70	15		5	5	1	5	40	0
4	4	1	4	99	1	3	99	0	0.0	21.6	94	110	70	10	t	4	4	1	4	10	0
5	5	1	5	40	0	1	35	0	0.0	30.0	98	130	80	15		5	5	1	5	45	0
5	5	1	5	40	0	1	10	1	0.0	26.4	102	110	70	15		5	5	1	5	50	0
3	3	2	3	99	1	3	99	1	0.0	90.0	104	120	70	10	t	3	3	2	3	99	1
4	4	1	4	99	1	3	99	1	0.0	78.5	94	140	90	10	t	5	5	1	5	19	9
4	4	1	4	20	1	3	99	0	0.0	74.0	96	120	70	10	t	4	4	1	4	30	1
4	4	1	4	10	0	1	10	0	0.0	0.0	92	100	70	15		5	5	1	5	20	0
5	5	1	5	30	0	1	10	0	0.0	25.0	104	100	60	15		5	5	1	5	30	0
4	4	1	4	99	1	2	99	0	0.0	22.0	84	100	60	15		4	4	1	4	20	0
4	4	1	4	30	0	1	30	0	0.0	18.5	100	130	80	15		5	5	1	5	30	0
4	4	2	4	99	1	3	99	0	0.0	49.0	102	100	80	10	t	4	4	1	4	99	1
3	3	2	3	99	1	2	99	0	0.0	68.0	86	110	70	15		4	4	1	4	20	0
4	4	1	4	15	1	2	99	0	0.0	203.0	104	100	60	15		5	5	1	4	20	0
4	4	2	4	99	1	3	99	0	0.0	49.0	100	110	70	5	T	4	4	2	4	99	1
3	3	2	3	99	1	3	99	1	0.6	136.6	95	120	70	7	t	3	3	2	3	99	1
4	4	1	4	18	1	1	30	0	0.0	15.0	90	110	70	15		5	5	1	5	24	0
5	5	1	5	20	0	1	30	0	0.0	12.0	94	120	80	15		5	5	1	5	14	0
3	3	2	3	99	1	3	99	1	0.0	124.0	94	140	90	10	t	3	3	2	3	99	1
5	5	1	5	55	0	1	17	0	2.0	47.5	92	130	80	15		5	5	1	5	30	0
3	3	2	3	99	1	3	99	0	0.0	45.0	78	102	78	10	t	4	4	2	4	10	1
3	3	2	3	99	1	3	99	0	0.0	45.0	78	108	78	10	t	5	5	1	5	10	1
4	4	2	4	99	1	2	99	0	0.0	28.2	92	100	80	10	t	5	5	1	5	99	1
5	5	1	5	30	0	1	22	0	0.0	40.0	108	100	70	14		5	5	1	5	30	0
5	5	1	5	99	1	2	99	0	0.0	64.0	102	130	90	10	t	5	5	1	5	99	1
4	4	2	4	99	1	3	99	1	0.0	161.5	100	130	80	10	t	5	4	1	5	99	1
5	5	1	5	30	0	1	28	0	1.2	18.2	84	120	70	15		5	5	1	5	20	0
3	3	2	3	99	2	3	99	1	204.0	204.0	88	120	80	10	t	4	4	2	4	99	1
5	5	1	5	38	0	1	30	0	1.2	26.2	84	100	60	15		5	5	1	5	48	0

VENTM	SBC3	CHOLC	ATRBOI	ATRINF	ATRTOT	HR4	SBP4	DBP4	GCS4	GCSI4	SHOULI	ELBOW	HAND4	HIP4	NHF4	RESPW	SBC4	MIOSIS	CREPTS	SAL4	DIARR4	CHOLC	ATRBOI
1	40	0	0.0	0.0	0.0	86	120	80	15		5	5	1	5	50	0	40	0	0	0	0	0	0.0
2	99	1	0.0	0.0	0.0	80	80	50	10	T	3	3	2	3	15	1	99	0	0	0	0	0	0.0
1	37	0	0.0	0.0	0.0	80	130	80	15		5	5	1	5	40	0	40	0	0	0	0	0	0.0
2	99	1	0.0	85.0	85.0	110	120	80	7	T	3	3	2	3	0	1	99	0	0	0	0	0	0.0
3	99	1	0.0	19.0	19.0	90	130	80	15		4	4	1	4	5	0	7	0	0	0	0	0	0.0
1	20	0	0.0	0.0	0.0	85	100	60	15		5	5	1	5	20	0	20	0	0	0	0	0	0.0
1	50	0	0.0	0.0	0.0	80	120	70	15		5	5	1	5	60	0	50	0	0	0	0	0	0.0
2	99	1	0.0	59.0	59.0	120	130	70	10	T	3	3	2	3	1	1	99	0	0	0	0	1	0.0
1	20	0	0.0	6.5	6.5	100	110	70	15		5	5	1	5	40	0	25	0	0	0	0	0	0.0
3	99	1	0.0	69.0	69.0	90	120	70	10	T	4	4	1	4	2	1	99	0	0	0	0	0	0.0
1	20	0	0.0	0.0	0.0	90	120	80	15		5	5	1	5	60	0	25	0	0	0	0	0	0.0
3	99	1	0.0	36.0	36.0	120	100	50	10	T	4	4	1	4	4	0	99	0	0	0	0	0	0.0
1	20	1	0.0	25.0	25.0	98	100	70	15		4	4	1	4	15	0	5	0	0	0	0	0	0.0
3	99	1	0.0	0.0	0.0	126	100	60	10	T	2	2	2	2	0	1	99	0	0	0	0	0	0.0
3	99	1	0.0	16.0	16.0	105	130	80	10	T	3	3	1	3	0	1	99	0	0	0	0	0	0.0
1	40	0	0.0	24.0	24.0	80	100	60	15		5	5	1	5	60	0	50	0	0	0	0	0	0.0
3	99	1	0.0	545.0	545.0	107	130	70	2	T	1	1	2	1	99	1	99	0	0	0	0	1	0.0
2	99	1	0.0	74.0	74.0	70	140	70	10	T	4	4	1	4	10	1	99	0	0	0	0	0	0.0
2	99	0	0.0	3.0	3.0	100	120	70	9	t	2	2	2	2	99	1	99	0	0	0	0	0	0.0
1	30	0	0.0	85.0	85.0	90	120	80	15		5	5	1	5	40	0	30	0	0	0	0	0	0.0
1	15	0	0.0	48.0	48.0	94	110	70	15		5	5	1	5	30	0	40	0	0	0	0	0	0.0
2	7	0	2.0	0.0	2.0	106	100	70	15		5	5	1	5	20	0	60	0	0	0	0	0	0.0
1	10	1	0.0	77.0	77.0	102	120	70	10	t	3	3	2	3	4	1	99	0	0	0	0	0	0.0
1	30	0	2.4	0.0	2.4	98	100	70	15		5	5	1	5	40	0	30	0	0	0	0	0	1.8
3	99	0	0.0	52.0	52.0	100	120	80	10	t	5	5	1	5	20	1	99	0	0	0	0	0	0.0
0	50	0	0.0	0.0	0.0	.	.	.	.				.		.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.	.	.
1	20	0	0.0	3.3	3.3	88	100	70	14		5	5	1	5	40	1	26	0	0	0	0	0	0.0
1	30	0	0.0	0.0	0.0	88	100	70	15		5	5	1	5	30	0	32	0	0	0	0	0	0.0
2	99	0	0.0	46.0	46.0	81	140	80	10	t	5	5	1	5	20	0	99	0	0	0	0	0	0.0
.	38	0	.	.	.	98	100	60	15		5	5	1	5	45	0	36	0	0	0	0	0	.
2	99	0	0.0	12.0	12.0	98	110	70	15		5	5	1	5	15	0	12	0	0	0	0	0	0.0
1	30	0	0.6	4.0	4.6	94	130	80	15		5	5	1	5	40	0	32	0	0	0	0	0	.
1	20	0	0.0	0.5	16.6	84	100	70	15		4	4	1	4	30	0	20	0	0	0	0	0	0.0
3	99	1	0.0	2.5	2.5	102	130	80	5	t	2	2	2	2	99	1	99	0	1	0	0	0	0.0
3	99	0	0.0	48.5	0.0	94	120	80	10	t	5	5	1	5	10	1	99	0	0	0	0	0	0.0
3	99	0	0.0	0.0	0.0	94	120	80	10	t	5	5	1	5	26	1	99	0	0	0	0	0	0.0
1	18	0	0.0	0.0	0.0	84	100	70	15		5	5	1	5	28	0	26	0	0	0	0	0	0.0
1	20	0	0.0	12.0	12.0	102	100	60	15		5	5	5	1	30	0	34	0	0	0	0	0	0.0
1	10	0	0.0	0.0	0.0	82	100	70	15		5	5	1	5	28	0	36	0	0	0	0	0	0.0
1	30	0	0.0	2.0	2.0	100	130	80	15		5	5	1	5	30	0	40	0	0	0	0	0	0.0
2	99	0	0.0	12.0	12.0	100	100	80	10		4	4	2	4	10	0	99	0	0	1	0	0	0.0
1	18	0	0.0	0.0	0.0	88	100	70	15		5	5	1	5	30	0	28	0	0	0	0	0	0.0
1	10	0	0.0	90.5	90.5	100	100	60	15		5	5	1	5	38	0	28	0	0	0	0	0	14.0
2	99	0	0.0	28.5	28.5	104	120	80	7	T	3	3	2	3	99	1	99	0	1	0	0	0	0.0
3	99	0	0.0	42.5	42.5	104	120	70	9	t	3	3	2	3	99	1	99	0	0	0	0	0	0.0
1	32	0	0.0	10.5	10.5	94	110	70	15		5	5	1	5	32	1	40	0	0	0	0	0	0.0
1	20	0	0.0	24.0	24.0	82	120	80	15		5	5	1	5	26	0	20	0	0	0	0	0	0.0
3	99	0	0.0	52.0	52.0	96	150	90	10	t	3	3	2	3	99	2	99	0	1	0	0	0	0.0
1	20	0	0.0	59.5	59.5	104	130	80	15		5	5	1	5	44	0	28	0	0	0	0	0	0.0
2	99	0	0.0	3.0	3.0	100	102	78	15		5	5	1	5	20	1	15	0	0	0	0	0	0.0
3	99	0	0.0	9.0	9.0	100	140	80	15		5	5	1	5	20	1	15	0	0	0	0	0	0.0
2	99	0	0.0	24.0	24.0	94	140	80	10	t	5	5	1	5	15	1	99	0	0	0	0	0	0.0
1	32	0	0.0	0.0	0.0	94	110	70	15		5	5	1	5	30	0	36	0	0	0	0	0	0.0
2	99	0	0.0	4.5	4.5	98	130	90	15		5	5	1	5	10	0	10	0	0	0	0	0	0.0
2	99	0	0.0	31.5	31.5	98	130	80	10	t	5	5	1	5	10	1	0	0	0	0	0	0	0.0
1	24	0	0.6	0.0	0.6	68	110	70	15		5	5	1	5	40	0	32	0	0	0	0	0	0.0
3	99	0	0.0	156.0	156.0	88	120	80	10	t	4	4	2	4	99	1	99	0	0	0	0	0	19.0
1	34	0	2.0	13.5	15.5	84	100	70	15		5	5	1	5	44	0	32	0	0	0	0	0	0.0

ATRINF	ATRTO	HR5	SBP5	DBP5	GCS5	GCSI5	SHOULI	ELBOW	HAND5	HIP5	NHF5	RESPW	VENTM	SBC5	CHOLC	ATRBOI	ATRINF	ATRTO	HR6	SBP6	DBP6	GCS6	GCSI6	SHOULI	ELBOW
0.0	0.0	85	120	80	15		5	5	1	5	50	0	1	40	0	0.0	0.0	0.0	.	.	.	.			
0.0	0.0	85	110	70	10	T	3	3	1	3	0	1	2	99	0	0.0	0.0	0.0	80	130	80	15		4	4
0.0	0.0	.	.	.	.				.		.	.	.	.	.	.	.	.	.	.	.	.			
0.0	0.0	80	110	70	7	T	2	2	2	2	2	1	3	99	0	0.0	0.0	0.0	80	110	70	8	T	3	3
0.0	0.0	106	110	70	15		4	4	1	4	15	0	1	10	0	0.0	0.0	0.0	90	110	70	15		5	5
0.0	0.0	.	.	.	.				.		.	.	.	.	.	.	.	.	.	.	.	.			
0.0	0.0	80	120	80	15		5	5	1	5	60	0	1	50	0	0.0	0.0	0.0	.	.	.	.			
36.0	36.0	120	120	70	10	T	3	3	2	3	1	1	3	99	0	0.0	0.0	0.0	110	110	60	10	T	2	2
0.0	0.0	90	110	70	15		5	5	1	5	40	0	1	25	0	0.0	0.0	0.0	.	.	.	.			
1.0	1.0	100	120	70	10	T	5	5	1	5	40	0	2	99	0	0.0	0.0	0.0	90	120	70	15		5	5
0.0	0.0	84	120	80	15		5	5	1	5	60	0	1	30	0	0.0	0.0	0.0	80	120	80	15		5	5
0.0	0.0	100	100	60	10	T	4	4	1	4	15	0	2	99	0	0.0	0.0	0.0	120	100	60	15		5	5
1.0	1.0	80	100	70	15		5	5	1	5	15	0	1	10	0	0.0	0.0	0.0	80	100	70	15		5	5
0.0	0.0	88	100	60	10	T	4	4	1	4	5	1	3	99	0	0.0	0.0	0.0	88	100	60	10	4	4	4
0.0	0.0	88	100	70	10	T	4	4	1	4	5	1	3	99	0	0.0	0.0	0.0	96	110	70	10	T	4	4
5.5	5.5	58	100	60	15		5	5	1	5	60	0	1	54	0	0.0	9.5	9.5	76	100	60	15		5	5
460.0	460.0	100	130	70	2	T	1	1	2	1	99	1	3	99	0	0.0	0.0	0.0	.	.	.	.			
20.0	20.0	85	130	90	10	T	4	4	1	4	30	1	2	99	0	0.0	0.0	0.0	88	130	80	15		4	4
0.0	0.0	100	120	70	9	t	2	2	2	2	0	2	3	0	0	0.0	0.0	0.0	108	130	80	9	t	2	2
20.0	20.0	90	120	80	15		5	5	1	5	40	1	1	30	0	0.0	0.0	0.0	88	120	80	15		5	5
21.0	21.0	98	110	70	15		5	5	1	5	30	0	1	40	0	0.0	5.0	5.0	86	110	70	15		5	5
0.0	0.0	108	100	70	15		5	5	1	5	20	0	1	60	0	0.0	0.0	0.0	104	100	70	15		5	5
84.0	84.0	96	120	70	10	t	3	3	1	3	4	1	3	99	0	0.0	15.8	15.8	90	120	70	10	t	4	4
0.0	1.4	102	100	70	15		5	0	1	5	40	0	1	30	0	1.8	0.0	1.8	92	100	70	15		5	5
0.0	0.0	90	120	70	15		5	5	1	5	45	0	1	10	0	.	.	.	90	120	80	15		5	5
.	.	.	.	.	.				.		.	.	.	.	.	.	.	.	.	.	.	.			
.	.	.	.	.	.				.		.	.	.	.	.	.	.	.	.	.	.	.			
0.0	0.0	88	100	70	15		5	5	1	5	40	0	1	26	0	0.0	0.0	0.0	.	.	.	.			
0.0	0.0	86	100	70	15		5	5	1	5	30	0	1	30	0	.	.	.	.	.	.	.			
24.0	24.0	80	100	70	15		5	5	1	5	20	0	1	5	0	0.0	7.0	7.0	88	100	70	10	t	3	3
.	.	84	100	70	15		5	5	1	5	50	0	1	40	0	0.0	0.0	0.0	.	.	.	.			
0.0	0.0	92	110	70	15		5	5	1	5	45	0	1	.	0	0.0	0.0	0.0	88	110	70	15		5	5
.	.	82	130	90	15		5	5	1	5	30	5	1	40	0	0.0	.	.	80	130	90	15		5	5
1.0	12.0	78	100	70	15		3	3	1	3	15	0	1	14	0	0.0	1.0	1.0	98	100	70	10	t	3	3
0.0	0.0	98	120	80	5	t	2	2	2	2	99	1	3	99	0	0.0	0.0	0.0	98	120	80	4	t	2	2
8.5	8.5	98	130	80	10	t	5	5	1	5	15	1	2	99	0	0.0	0.0	0.0	94	130	80	10	t	5	5
0.0	0.0	92	120	70	15		5	5	1	5	35	0	1	20	0	0.0	0.0	0.0	92	120	80	15		5	5
0.0	0.0	86	100	70	15		5	5	1	5	38	0	1	40	0	0.0	0.0	0.0	.	.	.	.			
1.5	1.5	108	100	70	15		5	5	1	5	30	0	1	30	0	0.0	0.0	0.0	98	100	60	15		5	5
0.0	0.0	84	100	70	15		5	5	1	5	46	0	1	38	0	0.0	0.0	0.0	.	.	.	.			
0.0	0.0	94	130	80	15		5	5	1	5	30	0	1	46	0	0.0	0.0	0.0	82	130	80	15		5	5
62.0	62.0	102	100	70	10	t	5	5	1	5	15	1	2	99	0	0.0	56.0	56.0	102	100	70	15		5	5
0.0	0.0	84	100	70	15		5	5	1	5	30	0	1	24	0	0.0	0.0	0.0	86	100	70	15		5	5
14.0	14.0	102	100	70	15		5	5	1	5	40	0	1	30	0	0.0	0.0	0.0	94	100	70	15		5	5
61.0	61.0	94	150	90	6	T	3	3	2	3	99	1	2	99	0	0.0	62.0	62.0	94	140	80	4	T	3	3
12.0	12.0	92	138	78	3	t	2	2	2	2	99	1	2	0	0	0.0	20.0	20.0	100	110	78	6	t	3	3
12.0	12.0	86	110	70	15		5	5	1	5	34	0	1	36	0	0.0	2.0	2.0	88	110	70	15		5	5
24.0	24.0	86	120	80	15		5	5	1	5	30	0	.	40	0	0.0	4.0	4.0	84	120	80	15		5	5
14.0	14.0	112	140	90	10	t	4	4	2	4	5	1	3	99	0	0.0	24.0	24.0	981	140	90	10	t	3	3
81.0	81.0	80	130	80	15		5	5	1	5	46	0	1	30	0	0.0	6.0	6.0	100	130	80	15		5	5
12.0	12.0	88	150	70	15		5	5	1	5	20	0	1	15	0	0.0	2.5	2.5	88	140	90	15		4	4
12.0	12.0	88	150	90	15		`5	5	1	5	16	0	1	15	0	0.0	2.5	2.5	88	140	90	15		5	5
21.0	21.0	96	106	80	10	t	3	3	2	3	4	0	3	99	0	0.0	12.0	12.0	92	100	70	10	t	3	3
0.0	0.0	86	100	70	15		5	5	1	5	40	0	1	36	0	0.0	0.0	0.0	.	.	.	.			
0.0	0.0	84	100	70	15		5	5	1	5	20	0	1	24	0	0.0	0.0	0.0	86	110	70	15		5	5
12.0	12.0	106	130	80	15		5	5	1	5	10	0	1	14	0	7.0	7.0	7.0	100	120	80	15		5	5
0.0	0.0	112	120	70	15		5	5	1	5	48	0	1	38	0	0.0	0.0	0.0	.	.	.	.			
19.0	19.0	981	120	70	10	t	4	4	1	4	10	1	2	99	0	0.0	0.0	0.0	74	100	60	10	t	4	4
5.5	0.0	72	100	80	15		5	5	1	5	44	0	1	32	0	0.0	0.0	0.0	98	100	80	15		5	5

HAND6	HIP6	NHF6	RESPW	VENTM	SBC6	CHOLC	ATRBO	ATRINF	ATRTO	HR7	SBP7	DBP7	GCS7	GCSI7	SHOULI	ELBOW	HAND7	HIP7	NHF7	RESPW	VENTM	SBC7	CHOLC	ATRBO	ATRINF
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
1	4	10	1	4	99	0	0.0	0.0	0.0	96	110	60	15		4	4	1	4	10	0	1	20	0	0.0	0.0
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
1	3	2	1	3	99	0	0.0	0.0	0.0	80	110	70	8	T	3	3	1	3	15	1	3	99	0	0.0	0.0
1	5	15	0	1	15	0	0.0	0.0	0.0	80	110	70	15		5	5	1	5	15	0	1	15	0	0.0	0.0
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
2	2	0	1	3	99	0	0.0	0.0	0.0	112	110	70	10	T	3	3	2	3	0	1	3	99	0	0.0	0.0
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
1	5	45	0	1	10	0	0.0	0.0	0.0	90	120	70	15		5	5	1	5	60	0	1	20	0	0.0	0.0
1	5	60	0	1	30	0	0.0	0.0	0.0	80	120	80	15		5	5	1	5	60	0	1	30	0	0.0	0.0
1	5	60	0	1	50	0	0.0	0.0	0.0	90	100	60	15		5	5	1	5	60	0	1	60	0	0.0	0.0
1	5	60	0	1	22	0	0.0	0.0	0.0	.	.	.	.				.		.	.	.	.	.	.	.
1	4	15	1	3	99	0	0.0	0.0	0.0	90	100	60	10	T	4	4	1	4	15	1	3	99	0	0.0	0.0
1	4	99	1	3	99	0	0.0	0.0	0.0	96	110	70	10	T	3	3	1	3	0	1	3	99	0	0.0	0.0
1	5	60	0	1	54	0	0.0	1.0	1.0	.	.	.	.				.		.	.	.	.	.	.	.
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
1	4	30	0	1	10	0	0.0	0.0	0.0	90	130	70	15		4	4	1	4	30	0	1	15	0	0.0	0.0
2	2	0	1	3	99	0	0.0	0.0	0.0	110	130	80	9	t	2	2	2	2	0	1	3	99	0	0.0	0.0
1	5	45	0	1	35	0	0.0	0.0	0.0	84	120	80	15		5	5	5	5	45	0	0	30	0	0.0	0.0
1	5	30	0	1	40	0	0.0	0.0	0.0	88	110	70	15		5	5	1	0	30	0	1	46	0	0.0	0.0
1	5	30	0	1	60	0	0.0	0.0	0.0	.	.	.	.				.		.	.	.	.	.	.	.
2	4	5	1	2	99	0	0.0	0.0	0.0	92	120	70	15		5	5	1	5	12	0	1	10	0	0.0	0.0
1	5	45	0	1	30	0	0.0	0.0	0.0	88	100	70	15		5	5	1	5	45	0	1	40	0	0.0	0.0
1	5	45	0	1	39	0	0.0	.	.	.	.	.	.				.		.	.	.	.	.	.	.
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
2	3	0	1	2	99	0	0.0	0.0	0.0	86	100	70	10	t	3	3	2	3	5	1	2	99	0	0.0	0.0
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
1	5	45	1	1	40	0	0.0	0.0	0.0	84	110	70	15		5	5	1	5	40	0	1	42	0	0.0	0.0
1	5	45	0	1	40	0	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
2	3	5	1	3	99	0	0.0	0.0	0.0	100	100	70	10	t	3	3	2	3	5	1	3	99	0	0.0	0.0
2	2	99	1	3	99	0	0.0	6.0	6.0	1	130	80	10	t	3	3	2	2	99	2	1	99	0	0.0	23.0
1	5	20	1	2	99	0	0.0	0.0	0.0	110	130	80	10	t	5	5	1	5	20	0	4	14	0	0.0	0.0
1	5	40	0	1	30	0	0.0	0.0	0.0	94	120	70	15		5	5	1	5	40	0	1	36	0	0.0	.
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
1	5	30	0	1	42	0	0.0	0.0	0.0	.	.	.	.				.		.	.	.	.	.	.	.
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
1	5	30	0	1	46	0	0.0	0.0	0.0	.	.	.	.				.		.	.	.	.	.	.	.
1	5	20	1	1	10	0	0.0	5.0	5.0	98	100	70	15		5	5	1	5	28	0	1	24	0	0.0	0.0
1	5	40	0	1	36	0	0.0	0.0	0.0	82	100	70	15		5	5	1	5	60	1	1	40	0	0.0	0.0
1	5	48	0	1	40	0	0.0	0.0	0.0	.	.	.	.				.		.	.	.	.	.	.	.
2	3	99	1	2	99	0	0.0	72.0	72.0	86	118	86	7	T	3	3	2	3	99	1	2	99	0	0.0	36.0
2	3	99	3	2	99	0	0.0	46.0	46.0	118	116	78	8	t	3	3	2	3	99	1	2	99	0	0.0	38.0
1	5	36	0	1	42	0	0.0	0.0	0.0	.	.	.	.				.		.	.	.	.	.	.	.
1	5	36	0	1	44	0	0.0	0.0	0.0	.	.	.	.				.		.	.	.	.	.	.	.
2	3	10	1	3	99	0	0.0	21.0	21.0	92	140	90	10	t	3	3	2	3	10	1	3	99	0	0.0	3.0
1	5	48	0	1	38	0	0.0	0.0	0.0	100	100	70	13	8	5	5	1	5	47	0	1	40	0	0.0	0.0
1	4	20	0	1	14	1	58.7	38.0	96.7	84	140	90	15		4	4	1	4	20	0	1	20	1	0.0	119.0
1	5	20	0	1	14	1	58.7	86.0	144.7	84	140	90	15		5	5	1	5	22	0	1	20	1	0.0	119.0
2	3	4	1	3	99	0	0.0	0.0	1.5	94	100	70	10	t	3	3	2	3	99	1	2	99	0	0.0	0.0
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
1	5	30	0	1	28	0	0.0	0.0	0.0	98	120	70	15		5	5	1	5	40	0	1	36	0	0.0	0.0
1	5	18	0	1	24	0	0.0	0.0	0.0	98	130	80	15		5	5	1	5	30	0	1	32	0	0.0	0.0
.		.	.	.	.	.	.	.	.	.	.	.	.				.		.	.	.	.	.	.	.
2	4	15	1	2	0	0	0.0	0.0	0.0	78	120	80	10	t	4	4	2	4	15	1	2	99	0	0.0	0.0
1	5	42	0	1	38	0	0.0	0.0	0.0	.	.	.	.				.		.	.	.	.	.	.	.

ATRTO	SED7	INSYN	INSYN	ICU	ICUDU	VENT	VENTL	TRACH	CMCG	CMCC	CMCA	ATRDU	ATRDO	DEATH	OUTC	HOSPI	DOPE	BCHEI	ACHEI	BCHEI	ACHEI	BCHEI	ACHEI	BCHEI	ACHEI
.	.	0		0		0			1	1	0			0	1	5	0	459.2	31.5	328	47.6	1061	36	1579	20.49
0.0	0	1	3	1	6	1	5	0	1	0	1	2	181.8	0	1	9	0	360.3	3.9	88	19.25	41.3	1.7	80	11.33
.	.	0		1	2	1	1.2	0	1	0	1	1	63	0	1	4	0	428.3	0.4	151	11.21	763.5	6.3	574	21.86
0.0	0	1	5	1	9	1	8	0	1	1	1	3	376.2	0	1	11	0	2020	34.7	9712	45.95	3499	45.1	6637	75.79
0.0	0	0		1	3	1	2	0	1	0	1	3	225	0	1	7	0	1007	16.8	758	52.57	1036	19.9	2141	62.71
.	.	0		0		0			1	1	1	1	0.6	0	1	5	0	1632	56.9	4379	125.5	1895	63	3136	97.19
.	.	0		0		0			1	0	1	2	23.8	0	1	5	0	1856	20.4	2915	18.6	993.2	12.6	721	8.04
0.0	0	1	9	1	16	1	14	0	0	0	1	4	326.7	0	1	19	0	459.4	0	126	0	747.5	0	369	4.84
.	.	0		0		0			1	1	1	3	30.8	0	1	5	0	1291	18.5	2657		996.2	11.2	2230	21.98
0.0	0	0		1	7	1	6	0	0	0	1	4	853.5	0	1	8	0	713.3	0	75	3.79	814.7	2.5	125	3.35
0.0	0	0		1	4	1	2	0	1	1	1	2	51	0	1	7	0	2517	50.7	5145	97.93	2182	34.7	4899	112.4
0.0	0	0		1	6	1	6	0	1	0	1	3	114	0	1	8	0	749.5	2.6	500	4.07	833.4	0.2	462	1.77
.	.	0		0		0			0	0	1	4	72.3	0	1	6	0	689.2	17.2	4314		889.3	2	55	25.52
0.0	0	1	8	1	11	1	11	0	1	1	1	2	190.3	1	2	11	0	537	0	54	8.69	376.1	0	55	0
0.0	0	1	10	1	16	1	13	1	0	0	1	3	118.9	0	1	27	0	459.3	0	41	0	446.6	0	38	7.95
.	.	0		0		0			1	0	1	6	69.2	0	1	6	0	1294	21.2	1314	9.84	779.9	5.2	727	5.99
.	.	0		1	5	1	5	0	0	0	1	4	2073	1	2	5	0	571.5	1.1	44	8.07	345.8	0.4	53	3.32
0.0	0	1	2	1	7	1	6	0	0	0	1	4	592	0	1	9	0	543.9	0	280	4.44	830.8	1.7	578	1.33
0.0	1	1	19	1	21	1	19	1	1	1	1	3	186.6	0	1	26	0	585.8	0.8	71	2.2	539.8	0.1	85	0.53
0.0	0	0		0		0			1	1	1	3	207.1	0	1	7	0	432.9	21.4	2018	14.5	153.3	2.3	91	3.04
0.0	0	0		0		0			1	1	1	4	167	0	1	6	0	717.9	2.2	73	16.95	980.9	1.1	130	1.21
.	.	0		1	2	0			1	1	1	2	18	0	1	6	0	697.4	17.1	65	13.13	715.8	13.2	64	7.73
0.0	0	1	4	1	4	1	3	0	0	1	1	5	242.8	0	1	7	0	755.5	9	44		541.8	1.3	53	8.44
0.0	0	0		0		0			1	1	1	4	7.8	0	1	7	0	2639	43.5	7362	56.42	2743	36.9	6222	50.89
.	.	0		1	4	1	3	0	1	1	1	3	205.2	0	1	6	0	657.3	1.4	118	12.45	635.4	0	122	9.38
.	.	0		0		0			1	1	0			0	1	3	0	2396	62.1	4238	45.46	2271	47.2	4757	
.	.	0		0		1	0.1	0	1	1	1	1	33.8	1	2	1	0	1086	1.2	1407	1.15				
.	.	0		0		0			1	1	1	3	111.9	0	1	5	0	614.2	1.2	110	5.28	554.6	7.7	114	13.29
.	.	0		0		0			1	1	1	2	21	0	1	5	0	3191	27.5	6828	50.3	2585	33.7	6429	55.02
0.0	1	1	6	1	10	1	11	1	1	1	1	4	267	0	1	19	0	512.1	1.1	109	9.35	550.9	1	85	6.67
.	.	0		0		0			1	1	1	1	33.8	0	1	5	0	709.9	13.5			580.4	10.8		
0.0	0	0		1	4	1	3	0	1	1	1	3	88.6	0	1	7	0	1754	7.8			3342	29.8		
.	.	0		0		0			1	1	1	3	87.6	0	1	6	0	945	5.7			856.9	4.3		
0.0	0	1	6	0		1	5	0	1	1	1	4	56	0	1	14	0	245.1	7.4			209.8	2.6		
23.0	0	1	25	1	25	1	25	1	1	1	1	9	555.5	0	3	25	1	436.5	1.9			458.6	1.7		
0.0	.	0		1	7	1	5	0	1	1	1	3	137.3	0	1	11	0	401.5	1			385	1		
0.0	0	0		1	5	1	4	0	1	1	1	26	0.6	0	1	8	0	424.5	2.4			239.5	3.3		
.	.	0		1		0			1	1	1	1	21	0	1	5	0	688.4	13.8			734	13.5		
.	.	0		0		0			1	1	1	4	72.5	0	1	6	0	531.8	3.9			437.5	2.4		
.	.	0		1	3	1	2	0	1	1	1	2	113	0	1	5	0	324	4.3			366	4.1		
.	.	0		0		0			1	1	1	3	28.5	0	1	6	0	677	21.4			646	20.7		
0.0	0	0		1	7	1	5	0	1	1	1	6	742.4	0	1	11	0	450.7	0.1			580.9	0		
0.0	0	0		1	4	1	2	0	1	1	1	2	228	0	1	7	0	599.2	0.3			607.1	4.2		
.	.	0		1	4	1	2	0	1	1	1	4	561.3	0	1	6	0	482.4	1.7			485.7	1.7		
36.0	0	1	22	1	27	1	26	1	1	1	1	18	666	0	1	30	1	691	9.5			682.5	0.7		
38.0	0	1	5	1	11	1	10	0	1	1	1	9	372.5	0	3	11	0	916	3.2			733	2.1		
.	.	0		0		0			1	1	1	5	62.8	0	1	6	0	716.7	34.8			689.9	31.7		
.	.	0		0		0			0	0	1	5	99	0	1	6	0	631.8	4.9			784.3	4.7		
3.0	0	1	5	1	9	1	8	1	1	1	1	6	278	0	3	9	0	487.5	0.3			642.8	0		
0.0	0	0		0		0			1	1	1	5	202	0	1	5	0	471.5	0.2			547.6	0.4		
119.0	0	1	3	1	9	1	8	0	0	1	1	11	762.7	0	1	14	0	914.8	2.7			698.6	2.1		
119.0	0	1	2	1	7	1	6	0	1	1	1	10	763.7	0	1	14	0	827	0.9			418.2	1.8		
0.0	0	1	5	1	12	1	12	1	1	1	1	6	149.2	0	1	15	0	816.8	1.6			469.6	0		
.	.	0		0		0			1	1	1	2	70	0	1	5	0	856.4	8			686.4	7.4		
0.0	0	0		1	4	1	3	0	1	1	1	3	140.5	0	1	7	0	689.7	2.4			696.8	0.5		
0.0	0	0		1	6	1	4	0	1	1	1	4	264.5	0	1	7	0	867.9	0			555.2	2.3		
.	.	0		0		0			1	1	1	2	33.6	0	1	5	0	490.2	0			607.6	1.9		
0.0	0	0		1	11	1	8	0	1	1	1	4	503	0	1	14	0	980.7	1.9			1211	0		
.	.	0		0		0			1	1	1	3	48.3	0	1	6	0	811.6	43.6			734.9	37.5		

BCHE	ACHE	BCHE	ACHE	BCHE	ACHE	BCHE	ACHE	BCHE	ACHE	BCHE	ACHE	BCHE	ACHE	BCHE	ACHE	BCHE	ACHE	BCHE	ACHE	BCHE	ACHE	BCHE	ACHE
1936	38.4	3110	13.32	1934	35.4	4004	64.27	1088	24.7	4691	63.65												
0	0	99	14.56	0	0	70	17.26	0	0		10.93	631.5	6.4	725	15.21	803	6.6	1156	26.45	971.8	12	1848	28.04
1011	11.5	1334	29.98	1596	18.1	2312	28.88																
3030	42.6	6826	99.05	2812	42.6	6457	57.01	2734	43.2	5470	148.3	2361	45.3	5706	107.3	2980	47.3	6219	146.9	3225	47.4	6442	97.4
1223	23.4	2167	47.41	1533	25.52	2591	53.84	1407	25.3	2869	46.42	1680	25.9	3443	50.02	1984	25.4	4379	51.7				
1854	54	3001	117.3	1495	55.3	2867	125.5																
707.1	7.1	271	7.64	835.5	7	316	36.23																
963.9	1.4	1119	4.53	1192	1.7	1485	5.53	1280	2.2	1789	0	875	2.8	1096	9.87	996.2	4.1	1395	8.6				
1121	12.1	2046	49.77	1537	12	2603	54.49	1550	9.4	3408													
877.6	0	165	3.91	947.7	0	331	9.94	699	0	395	6.61	571.7	0.1	354	6.96	766.2	0	559	6.15	1035	2.6	849	
2234	51.8	4314	79.61	2192	47.2	3695		1727	36.8	4237	96.53	2504	49.1	4531	98.49	2187	50	5201	130.7	2453	51.1	5314	99.2
539.5	1.6	234	12.36	305.1	1.2	508	13.91	677.4	0	993	13.2	957.7	2.4	1317	15.34	1156	3.3	1968	12.92	1095	3.1	2422	9.25
507.6	1.6	43	15.06	362.2	7.7	43	17.48	535.5	9.1	127	17.85	666.6	11.4	434	35.12								
629	0.7	44	5.84	361	0.6	37	9.59	534.6	2.3	39	5	367.8	2.8	59		332.5	1.3	48	8.6	335	1.4	95	8.94
505.8	0.8	37	6.21	601.2	1.2	54	4.32	47	1.4	106	16.08	558.1	0.3	247	8.66	572.2	0.7	689	10.21	598.1	0.3	1197	0
758.8	3.3	663	8.85	1316	4.4	35	11.43	2913	49.7	7128	42.63	1149	7.1	1861	11.05	977.7	6.2	2357	29.03				
80.1	0.7	191	3.91	464.5	0	596	8.07	729.5	0	796	5												
1149	1.7	1014	0	1082	1.8	1367	0.78	1450	2.1	1996	1.18	1317	3.4	2411	7.73	1352	3.1	2242	4.35	1640	4.3	2684	7.17
721.6	0.3	35	1.65	944.3	2.8	38	0	800.1	1.9		5.37	980.1	2.8	45	0	410	2.3	54	0	427	1.9	102	1.65
526.9	1.7	59	4.1	717.9	3.1	47	0	477.7	2.8	51	0.12	644.3	0.8	54	0.56	482.3	3.1	6	3.91				
367	2.6	404	0.09	718.9	2.9	150	2.02	1080	3.1	229	2.39	733.2	4.3	526		1112	3.9	836	3.32				
185.2	4.3	68	0.9	812.9	6.6	53	6.08	541.2	9.7	97		705	11.4	101	8.51								
514	1.4	73	4.16	605.3	2.4	43	2.64	620	2.5	63	0	641	3.1	8	0.99	680.3	3.5	60	0	580.2	1.6	31	
3288	41.1	6372	66.14	2591	41.8	6570	61.98	2697	44	5307													
563.8	4.3	165	8.23	578.1	2	63	2.08	583.6	3.6	86	6.08	867.2	3.7		4.53								
2542	33.7		55.49																				
583.2	7.3	151	1.43	782.8	13.5	402	25.24																
2712	30.4	6171	53.47	2875	29.9	6607	59.68	2876	36.2	6083	52.2	2565	35		55.55								
639.5	0.8	87	0	708	1.7	76	3.82	841.6	3.2	66	0	575.3	4.7	43	3.38	584.5	1.1	58	3.2	418.1	1		5.74
716.6	12.4			684.3	21.6			957	31														
2981	44.5			2856	44.1			2352	46.4			4035	42.6			3848	45						
759	4.3			935.7	0.9			981	4.6			1369	1.5			1509	4.4						
284.3	2.5			140.6	2.7			316.4	2.1			242.3	5.1			186.2	4.6			177.8	4.1		
322.1	0.7			437.6	1.8			416.4	1.7			473.8	1.6			585.7	4.1			490.8	1.5		
460.4	1.9			271.2	2.1			432.6	2.9			301.8	3.1			463	1.8			612.8	2.3		
340.4	4			311.9	4.5			352.8	7.1			522.8	8.8			513.7	9.7						
1109	12.4			1365	16.1																		
604.8	3.6			637.2	7.8			335	19.3			399	22.9										
326.6	6.7			251.7	9			397.4	9.6														
544.2	27.1			785.3	21.6			1139	22.3			1354	24.2										
298.1	7.4			583.9	0.3			761.3	0.1			985.6	0.2			1152	5.3			973.4	3.8		
896.5	7.3			941.3	17.1			915.1	12.4			1365	15.4										
670.1	0			587.3	1			549.1	2.7														
526	0.2			552.9	0.6			418.3	1.7			609.1	1			506.8	2.9			588.3	0		
644.1	9.3			617.6	13.7			634.8	15			822.8	12.4			845.6	14.8			1124	16		
860.5	37.8			1107	40.6			1181	34.8														
509.9	8.4			547.8	9.4			681.3	9.4														
1220	0.3			1225	1.3			1364	1.9			1548	2.6			1446	2			1769	1.5		
565	2.7			650	3.6			593	5.5			768.8	5.1										
602.6	1.3			458.6	2			483.2	3.8			493.8	2.3			665.5	1.7			541.4	4.2		
539.5	2.2			591.3	0.5			653.4	0.6			719.9	0.5			834.1	0.4			873.4	0.5		
418	1.5			862.5	1.4			1057	1.3			1088	2.6			1366	1.3						
711.8	5.2			464.8	4.6			605.8	6.7														
708.9	4			546.3	9.6			947.4	8.5			842.5	12.5			1164	12.8			1311	12.9		
602.3	0			698.8	0.6			660	1.2														
720.2	1.5			847.1	12.3			900.4	0.2														
1211	0			1820	0			979	2.2			849.4	1.2			1284	2.8			1148	1.9		
737	43.6			873.8	45.2			1259	42.7														